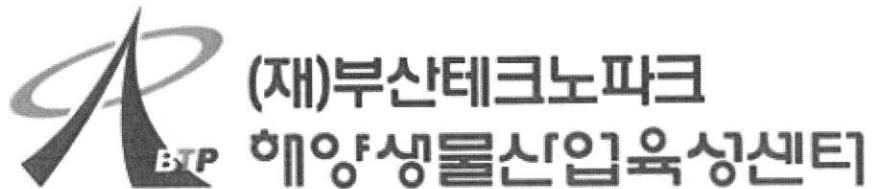


SHIPBOARD TEST REPORT

- Test Laboratory : Busan Techno-Park
Marine Bio-industry Development Center

- Test Period : 11. Sep. 2010 – 25. Mar. 2011



Shipboard Test Report

| | | | |
|-----------------|--|---------------|-----------------------|
| Test Laboratory | Busan Techno-Park Marine Bio-industry Development Center | | |
| Test Period | 11. Sep. 2010 – 25. Mar. 2011 | | |
| Test Number | Port(State) | Date | Success/Failure |
| SET-10-004 | New York(U.S.) | 12. Nov. 2010 | Success |
| SET-10-005 | Kaoshiung(Taiwan) | 18. Dec. 2010 | Success |
| SET-10-006 | Busan(Republic of Korea) | 24. Dec. 2010 | Success |
| Vessel type | Container ship | Vessel name | HANJIN DURBAN |
| Applicant | Samsung Heavy Industries Co.,Ltd. | Applied Model | Purimar SP-050 |
| Rated Capacity | 500 m ³ /hr | Pump Capacity | 500m ³ /hr |

■ Result

- The results of 3 consecutive, valid test cycles showing discharge of treated ballast water in compliance with regulation D-2.
- During shipboard test period, Samsung Heavy Industries Purimar™ Ballast Water Management System meets the standard set by regulation D-2 of IMO BWM Convention.

■ Attachment

- #1. Shipboard test SET-10-004 Test Plan / Report
- #2. Shipboard test SET-10-005 Test Plan / Report
- #3. Shipboard test SET-10-006 Test Plan / Report

Mar. 2011

Zip Code : 619-912

Address : 27, Hoenggye-ri, Ilgwang-myeon, Gijang-gun, Busan, Korea

Homepage : <http://www.btp.or.kr>

Phone : 82-51-723-3311

Fax : 82-51-723-3320

첨부(1)

시험번호 : SET-10-004

시험항구 : NEWYORK(U.S.A)

Test Plan

| | |
|---------------------|---|
| Project name | Purimar™ |
| Study number | TW-Ship(4) |
| Test number | SET-10-004 |
| Port | NEWYORK (U.S.A) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | JaeYoung Baek  (Sign) |

1. Test title

Efficacy test of Purimar™ Ballast Water Management System (shipboard scale)

2. Test purpose

2.1 The objective of the present test is to evaluate the efficacy of Purimar™ Ballast Water Management System(BWMS).

2.2 We will determine elimination efficacy of organism lager than 50 µm, organism between 10 and 50 µm and bacteria by treatment of Purimar™ BWMS.

3. Schedule

3.1 The expecting port of shipboard test : NEW YORK

3.2 Equipment usage(treatment) schedule

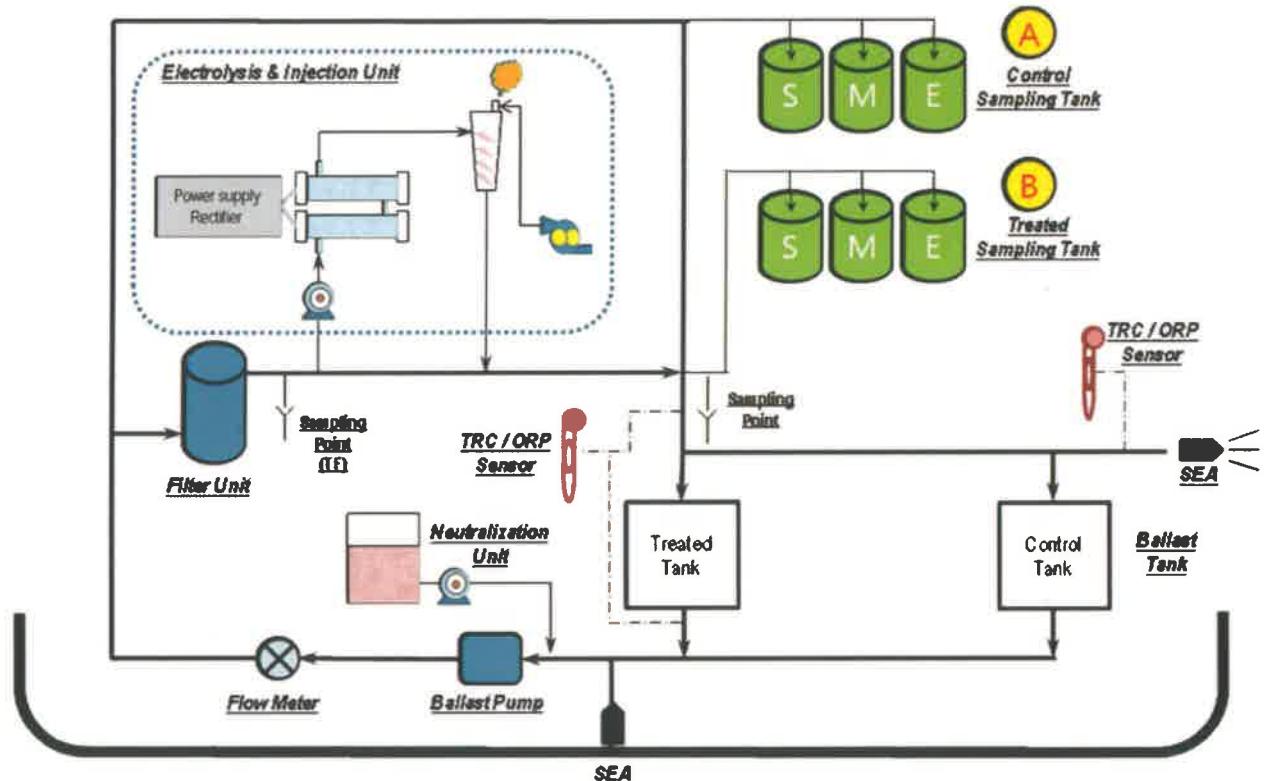
| Test time | Date | |
|-----------|--------------------|--------------|
| | Purimar™ Treatment | Deballasting |
| Day 0 | Nov 13, 2010 | - |
| Day 5 | - | Nov 18, 2010 |

3.3 Test schedule

| Test time | Date | | | | |
|-----------|---|------------------------------------|-----------------------------------|--------------------------------------|--|
| | * Water parameters * ≥50 µm Organisms * 10~50µm Organisms | * Coliform * Enterococcus group | * V. cholera | * Heterotrophic bacteria (Marine) | |
| Day 0 | Nov 13, 2010 | Nov 13, 2010 ~ Nov 14, 2010 | Nov 13, 2010 ~ Nov 15, 2010 | Nov 13, 2010 ~ Nov 16, 2010 | |
| Day 5 | Nov 18, 2010 | Nov 18, 2010 ~ Nov 19, 2010 | Nov 18, 2010 ~ Nov 20, 2010 | Nov 18, 2010 ~ Nov 21, 2010 | |

4. Sampling procedures

4.1 Sampling point



| Sampling point | Day | Sample name | Parameters |
|----------------|-------|---|--|
| A | Day 0 | Original water / Control (untreated) seawater | Water parameters ^{a)} Organism ^{b)} Bacteria ^{c)} |
| B | Day 5 | Treated ballast seawater | Water parameters ^{d)} Organism ^{b)} Bacteria ^{c)} |

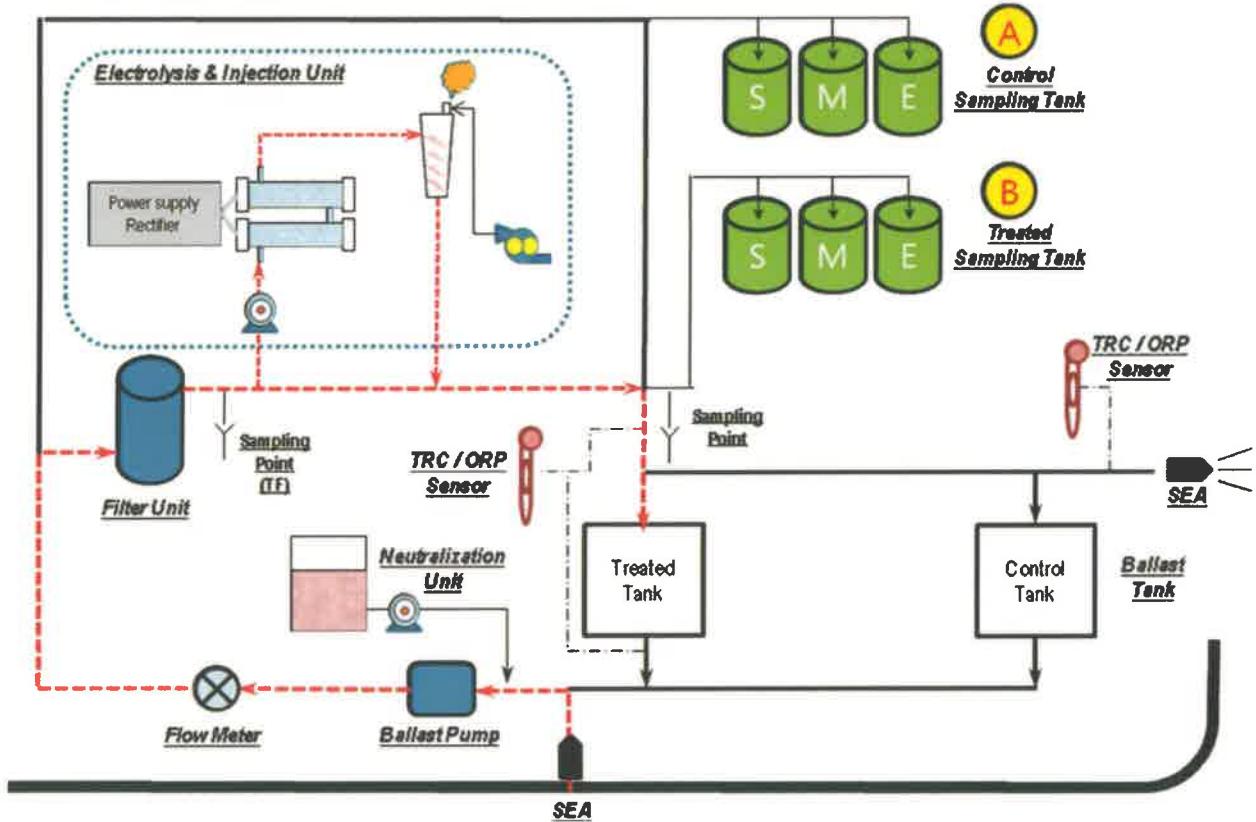
a) Water parameters: pH, Temperature, Salinity, DO, Turbidity, DOC, POC, TSS

b) Organism: $\geq 50 \mu\text{m}$ Organisms, $10\mu\text{m} - 50\mu\text{m}$ Organisms

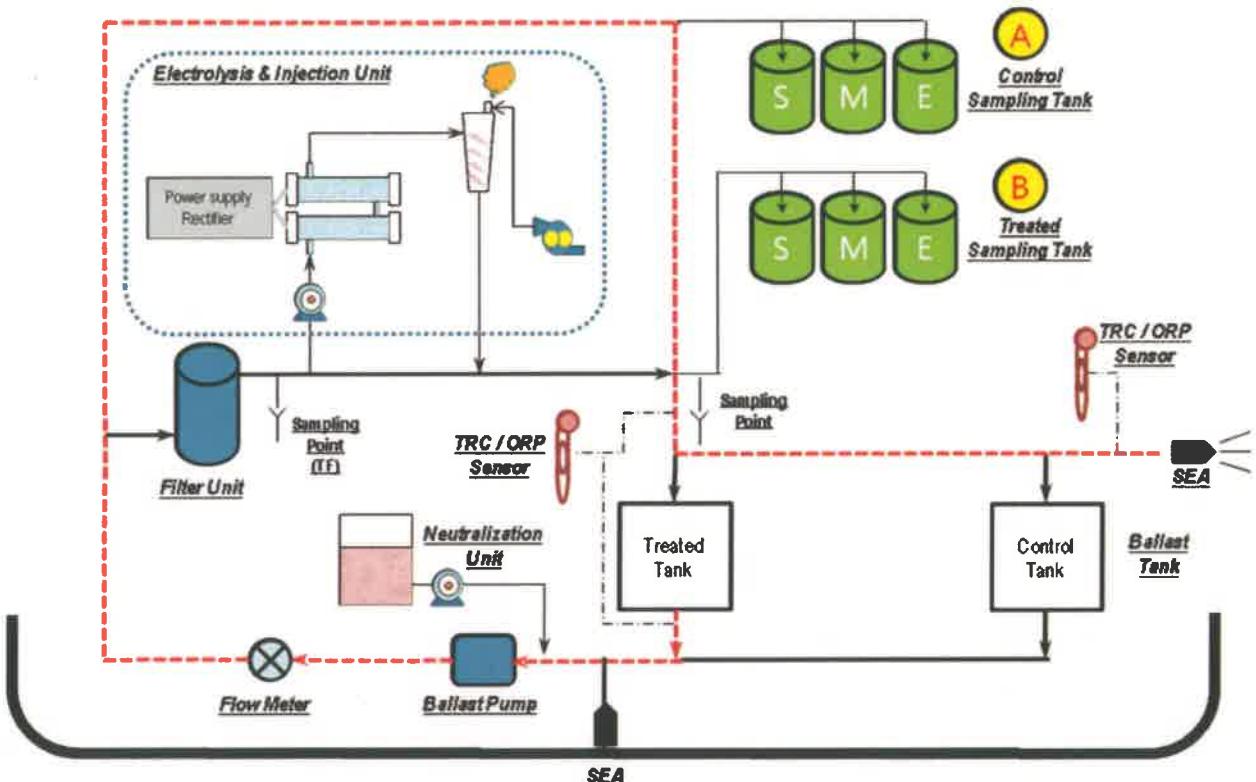
c) Bacteria: Heterotrophic bacteria, Coliform, Enterococcus group, *V. cholera*

d) Water parameters: pH, Temperature, Salinity, DO, Turbidity

4.2 Ballasting mode



4.3 Deballasting mode



4.4 Sampling procedures

- Collection and handling of field samples from the HANJIN “the DURBAN” will be undertaken by a team from MBDC and NLP Co., Ltd, using standard water sample collection methods and in accordance with the G8 Guidelines. Standard operating procedure (SOP) - SOP-BWMS-023 is employed to provide consistency and reproducibility to the sampling methods used by field personnel.
- Water samples will be taken from both the control (untreated) and treated tanks at two times intervals following treatment – at the ballasting (immediately after treatment, day 0), and at the discharge (deballasting or neutralizing agent treatment after six days, day 5), and identified as numbered sampling points.
- All sampling equipments, apparatus and containers are prepared in accordance with EPA’s Coastal 2000 Field Operation Manual. They are packed into exclusive cases.
- Water samples should be directly taken full up in a sample bottles after washing by sample water. When sample bottles are pre-preserved, the bottles should not be rinsed but be filled once with sample.
- Water parameters of samples are analyzed as soon as possible after collection at a field. The collected samples are transported to the laboratory in the DURBAN for the analysis.
- When the samples are arrived to laboratory, laboratory personnel receive the samples and entered the samples into the laboratory. The laboratory custodian will open the sample and carefully check the contents for evidence of leakage
- Sample handling will be performed so as to collect, store, submit to the laboratory and analyze representative samples using methods as specified in the test plans.

4.5 Test substances of Day 0

- Influent water (Original water)

| Parameter | Sampling point | Sample ID & Lot No. |
|------------------|----------------|---------------------|
| Organisms | A | OwS-1113 |
| Bacteria | | OwM-1113 |
| Water parameters | | OwE-1113 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T0S-1113 |
| Heterotrophic bacteria | | T0M-1113 |
| Water parameters | | T0E-1113 |

4.6 Test substances of Day 5

- Control (untreated) seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------|---------------|---------------------|
| Organisms | A | C5S-1118 |
| Bacteria | | C5M-1118 |
| Water parameters | | C5E-1118 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T5/S1, 2, 3-1118 |
| Heterotrophic bacteria | | T5/M1, 2, 3-1118 |
| Water parameters | | T5/E1, 2, 3-1118 |

5. Test procedures

5.1 Test design

| | |
|--------------------------|--|
| Test system | Purimar™ (shipboard scale) |
| Test substance | Original water: 100% Control (untreated) seawater: 100% Treated ballast seawater: 100% |
| Dilution water | Filtered natural seawater (FNS) |
| TRC concentration | Control: 0.0 ppm Treated: 3.0 ppm(\pm 0.5 ppm) |
| Sampling time | Day 0, 5 |

5.2 Test method

5.2.1 Water parameters measurement

- 1) Water parameters (temperature, pH, DO, salinity, turbidity) of samples at ship(HANJIN DURBAN) are measured using an MS5 according to SOP-BWMS-022.
- 2) Water parameters (DOC, POC) of samples at MBDC lab. are measured using vario TOC cube according to SOP-BWMS-021.
- 3) Water parameter (TSS) of samples at MBDC lab. is measured according to SOP-BWMS-005.

5.2.2 Biological efficacy test

- 1) $\geq 50\mu\text{m}$ organism
 - ① Concentration
 - Sample can be concentrated with 32 μm sieve.
 - After concentration, wash with filtered natural seawater to gather organisms.
 - Concentrated sample transfer into glass beaker and fill up to 100 mL with filtered natural seawater.
 - ② Analysis
 - General method
 - Analysis under stereo microscope with dark field (alive: movement /dead: lack of movement).
 - 1 ~ 20 mL of concentration sample place on a counting chamber (sedgewick-Rafter cell or Bogorov counting chamber).
 - The number of observations must be more than three.

- Analysis by process of dyeing
 - Making of FDA Stock solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
 - Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
 - Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
 - 1 mL of dyed sample place on a counting chamber.
 - Waiting for 10 minutes for cell staining.
 - Turn on mercury burner of microscope and apply to a fluorescent filter.
 - Viable cells represent green color.
 - Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

2) 10 μ m - 50 μ m organism

① General method

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- 0.1 mL of fixed sample place on a counting chamber (sedgewick-Rafter cell).
- Waiting for 5 minutes for cell sinking.
- The number of observations must be more than three.

② Staining

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- Making of FDA Stock Solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
- Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
- Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
- Dyed sample place on a counting chamber.
- Waiting for 10 minutes for cell staining.
- Turn on mercury burner of microscope and apply to a fluorescent filter.
- Viable cells represent green color.
- Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

3) Heterotrophic bacteria

- Standard: APHA 9215 (Heterotrophic plate count: 2005)
- SOP: SOP-BWTS-009
- Medium: Marine agar (DIFCO, Cat No 212185, Lot No 8129045)
- Method: Sample smear on the agar plate.
- Sample volume: 0.1 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 25°C, 3 days.
- Data analysis: Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates promptly after incubation. And calculate count per 1 mL.

4) *Escherichia coli*

- Standard: EPA 1603 [*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane - Thermo tolerant *Escherichia coli* Agar (Modified mTEC) : 2006]
- SOP: SOP-BWTS-010
- Medium: mTEC agar (DIFCO, Cat No 214880, Lot No 8171842)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Escherichia coli* (ATCC No #11775)
- Negative control: *Enterococcus faecalis* (ATCC No #19433)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: Incubate 35°C ± 0.5°C for 2 ± 0.5 hours. Transfer the plates to a Whirl-Pak® bag, seal the bag, and submerge in a 44.5°C ± 0.2°C water-bath for 22 ± 2 hours.
- Data analysis: Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL
- Report results as *E. coli* CFU per 100 mL of sample.

5) *Enterococcus faecalis*

- Standard: EPA 1600 [*Enterococci* in Water by Membrane Filtration Using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI): 2006]

- SOP : SOP-BWTS-011
- Medium: mEI agar (DIFCO, Cat No 214881, Lot No 8253196)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Enterococcus faecalis* (ATCC No #19433)
- Negative control: *Escherichia coli* (ATCC No #11775)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 41°C ± 0.5°C, 24 ± 2 hours
- Data analysis: Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL
- Report results as enterococci per 100 mL of sample.

6) *Vibrio cholerae* O1, O139

- SOP: SOP-BWTS-012
- Medium: TCBS agar (DIFCO, Cat No 265020, Lot No 8021353)
- Method
 - (1) Filter sample using 0.45 µm membrane filter and then place filters on the top of plate. Incubate the plates, protected from light at 35°C ± 1°C for 24 ± 2 hours.
 - (2) Each yellow sucrose fermenting colonies are placed on Non-salt Alkaline Pepton water. Incubate the plates at 36°C ± 1 °C for 6-18 hours.
 - (3) Presumptive positive colonies are performed using slide agglutination assays by O1 and O139 antiserum for serological identification.
- Sample volume: 100 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Data analysis: Select the colonies with agglutination O1 and O139 antiserum. Calculate the number of *Vibrio cholerae* per 100 mL
- Report results as *Vibrio cholerae* O1, O139 per 100 mL of sample.

6. Validity

6.1 Water parameters

6.1.1 Measurement of water parameters must be performed at least three times.

6.1.2 Sample should be analyzed as soon as possible after arrival at the DURBAN lab.

6.2 Biological efficacy test

6.2.1 Influent condition must be appropriate for the following IMO standards;

- The organism larger than 50 µm: $\geq 10^2$ individuals/m³
- The organism between 10 to 50 µm: $\geq 10^2$ individuals / mL,

6.2.2 Average discharge results from the control water is a concentration must be more than the values in regulation D2.1;

- The organism larger than 50 µm: ≥ 10 individuals/m³
- The organism between 10 and 50 µm: ≥ 10 individuals / mL

7. Data and report

7.1 Data

7.1.1 Water parameters measurement

- 1) Measurement of water parameters (temperature, pH, DO, salinity, Turbidity) are performed at least three times using measurement equipment (Model: MS5)
- 2) Measurement of water parameters (DOC, POC) are performed at least three times using measurement equipment (Model: vario TOC cube)

$$\text{POC} = \text{TOC-DOC}$$

- 3) Measurement of water parameter(TSS) is performed at least three times.

Calculate non-filterable residue as follows:

$$\text{Non-filterable residue (mg/L)} = \frac{(A - B) \times 1000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = mL of sample filtered

7.1.2 Biological efficacy test

Result data are presented mean value using calculation method as follows;

- 1) Survival rate

$$\text{Survival rate (\%)} = \frac{N_2}{N_1} \times 100$$

where:

N_1 = number of survival organism at the beginning

N_2 = number of survival organism at the end of the selected time interval

2) Heterotrophic bacteria

Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates. And calculate count per 1 mL.

$$\text{CFU/ mL} = \frac{\text{Number of colonies}}{\text{Volume of sample (mL)}}$$

3) *Escherichia coli*

Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$E \text{ coli / 100 mL} = \frac{\text{Number of } E. \text{ coli colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

4) *Enterococcus faecalis*

Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL according to the following general formula:

$$\text{Enterococci / 100 mL} = \frac{\text{Number of enterococci colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

5) *Vibrio cholera* O1, O139

Count positive results with slide agglutination assays by O1 and O139 antiserum for serological identification. Calculate the number of *Vibrio cholera* O1, O139 per 100 mL according to the following general formula:

$$Vibrio \text{ cholera O1, O139 / 100 mL} = \frac{\text{Number of } Vibrio \text{ cholera O1, O139}}{\text{Volume of filtered sample (mL)}} \times 100$$

7.1.3 Coefficient of variation (CV)

Coefficient of variation for each replicate should be calculated as follows.

$$CV (\%) = \frac{Y}{X} \times 100$$

where:

X: The mean value for respective replicate

Y: Standard deviation for respective replicate

7.2 Report

- QA statement
- Result
- Conclusion
- Appendix

Test Report

| | |
|--------------|--|
| Project name | Purimar™ |
| Study number | TW-Ship(4) |
| Test number | SET-10-004 |
| Port | NEWYORK (U.S.A) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | Jae Young, Back  <small>(Sign)</small> |

Statement of Quality Assurance

Data were reviewed by Quality Assurance Unit of DAU to assure that the study was performed in accordance with protocol and standard operating procedures (SOP) of Marine Bio-industry Development Center (MBDC). The report was an accurate reflection of the raw data generated at the MBDC. Inspection of the routine and repeated procedures that constitute the study was carried out as a continuous major phase at or about the time this study was in progress.

| Inspection Phase | | Date | | |
|--|-----------------|------------------|--------------------------|---------------------------|
| | | Inspection | Report to Study Director | Report to Project manager |
| Test plan | | Nov, 08, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| Sampling | 1 st | Nov, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Nov, 18, 2010 | | |
| Test substance | 1 st | Nov, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Nov, 18, 2010 | | |
| Test procedures (Bacteria test) | 1 st | Nov, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Nov, 18, 2010 | | |
| Observation and counting (≥50μm, 10μm-50μm) | 1 st | Nov, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Nov, 18, 2010 | | |
| Observation and counting (Bacteria test) | 1 st | Nov, 14, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Nov, 16, 2010 | | |
| | 3 rd | Nov, 19, 2010 | | |
| | 4 th | Nov, 21, 2010 | | |
| Raw data | | Nov, 26, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| Test report | | Mar, 23-25, 2011 | Mar, 28, 2011 | Mar, 28, 2011 |

Sang-Hee, Won (Sign)

Mar. 27. 2011

Sang-Hee, Won / Quality Assurance

Date

Study Personnel and Participants

The test participants recognized the study plan, manual, procedure, guide of Marine Bio-industry Development Center in performing the test.

| | Name | Date |
|---------------------------------|-----------------------|---------------|
| Study Personnel | | |
| | JaeTaung. Baek (sign) | Mar. 26. 2011 |
| | Jaewoo. Lee (sign) | Mar. 25. 2011 |
| | Han-na. Sung (sign) | Mar. 25. 2011 |
| | Gun-Jung. Jung (sign) | Mar. 25. 2011 |
| QA/QC | | |
| | Yeon Su. Park (sign) | Mar. 25. 2011 |
| Efficacy Testing Manager | | |
| | Ryong-Jin Ki (sign) | Mar. 27. 2011 |

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1. Results**1.1 Loading record**

| | |
|-------------|---------------|
| Vessel name | HANJIN DURBAN |
| IMO number | 9375513 |

| | Water ballast tanks | | Sample WGT (Mt) | Loading (Date/Location) | Discharge (Date/Location) |
|---------|---------------------|---|-----------------|-------------------------|----------------------------------|
| Control | No.6 D/B W.B.T | P | 433 | Nov, 13 New York | Nov, 18 27° -56.1N, 077-39.8W |
| Treated | No.6 D/B W.B.T | S | 433 | Nov, 13 New York | Nov, 18 27° 41.4N, 077-28.9W |

1.2 Water parameters

Table 1. Water parameters at field

| Date | Sample ID | pH | Temp (°C) | Salinity (‰) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------|------------|--------------|-----------|-----------------|--------------|
| Day 0 | OwS | 7.10±0.01 | 12.25±0.01 | 22.00±0.01 | 7.96±0.00 | 2.9±0.1 | 0.602±0.000 |
| | OwM | 7.22±0.01 | 11.53±0.01 | 22.12±0.01 | 7.81±0.01 | 2.7±0.1 | 0.652±0.000 |
| | OwE | 7.36±0.00 | 11.36±0.00 | 21.78±0.00 | 7.78±0.00 | 2.5±0.0 | 0.631±0.000 |
| | T0S | 7.35±0.01 | 12.01±0.01 | 22.46±0.00 | 7.42±0.01 | 1.1±0.1 | 0.000±0.000 |
| | T0M | 7.45±0.00 | 11.99±0.01 | 21.72±0.00 | 7.56±0.00 | 1.0±0.0 | 0.000±0.000 |
| | T0E | 7.51±0.00 | 11.96±0.00 | 22.13±0.01 | 7.35±0.01 | 1.3±0.1 | 0.000±0.000 |
| Day 5 | CSS | 7.19±0.01 | 26.45±0.01 | 23.70±0.00 | 7.84±0.00 | 3.2±0.1 | 0.106±0.000 |
| | C5M | 7.24±0.00 | 25.90±0.00 | 23.67±0.01 | 7.47±0.00 | 3.1±0.0 | 0.102±0.000 |
| | C5E | 7.39±0.01 | 25.86±0.01 | 23.66±0.01 | 7.43±0.00 | 3.0±0.1 | 0.105±0.000 |
| | T5/S1 | 7.73±0.00 | 25.23±0.00 | 23.08±0.01 | 7.03±0.00 | 1.4±0.0 | 0.000±0.000 |
| | T5/S2 | 7.79±0.01 | 24.99±0.01 | 23.11±0.01 | 6.82±0.00 | 1.5±0.0 | 0.000±0.000 |
| | T5/S3 | 7.93±0.00 | 25.11±0.01 | 23.11±0.01 | 6.95±0.00 | 1.8±0.0 | 0.000±0.000 |
| | T5/M1 | 7.93±0.00 | 25.12±0.00 | 23.11±0.00 | 6.96±0.00 | 1.8±0.0 | 0.000±0.000 |
| | T5/M2 | 7.96±0.00 | 24.95±0.00 | 23.10±0.00 | 6.85±0.00 | 1.1±0.0 | 0.000±0.000 |
| | T5/M3 | 7.98±0.00 | 25.07±0.01 | 23.10±0.00 | 6.91±0.00 | 1.5±0.0 | 0.000±0.000 |
| | T5/E1 | 8.01±0.00 | 25.23±0.00 | 23.12±0.00 | 7.03±0.00 | 1.6±0.1 | 0.000±0.000 |
| | T5/E2 | 8.01±0.01 | 25.16±0.01 | 23.16±0.00 | 6.98±0.00 | 1.0±0.1 | 0.000±0.000 |
| | T5/E3 | 8.02±0.00 | 25.32±0.00 | 23.16±0.00 | 7.09±0.00 | 1.5±0.1 | 0.000±0.000 |

Data were presented as mean±S.D of three repeated measurement.

Table 2. Results of DOC, POC, TSS

| Date | Sample ID | TOC ^{a)} (mg/L) | DOC ^{b)} (mg/L) | POC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | 2.76±0.03 | 1.70±0.04 | 1.06 | 6.7±0.4 |
| | OwM | 2.80±0.01 | 1.70±0.01 | 1.10 | 7.1±0.5 |
| | OwE | 2.78±0.01 | 1.72±0.02 | 1.06 | 6.7±0.3 |

Data were presented as mean±S.D of three repeated measurement.

a) TOC: Total Organic Carbon

b) DOC: Dissolved Organic Carbon

c) POC: Particulate Organic Carbon (POC=TOC-DOC)

d) TSS: Total Suspended Solids

Table 3. TRC decay

| Date | Measurement time | Control | Treated |
|--------------|------------------|----------------|----------------|
| | | TRC con. (ppm) | TRC con. (ppm) |
| Day 0 | 13:00 | 0.06±0.01 | 2.90±0.01 |
| Day 1 | 13:00 | 0.04±0.01 | 1.55±0.10 |
| Day 2 | 13:00 | 0.04±0.01 | 0.96±0.06 |
| Day 3 | 13:00 | 0.03±0.01 | 0.64±0.06 |
| Day 4 | 13:00 | 0.03±0.01 | 0.17±0.02 |
| Day 5 | 13:00 | 0.02±0.01 | 0.03±0.01 |

Data were presented as mean±S.D of three repeated measurement.

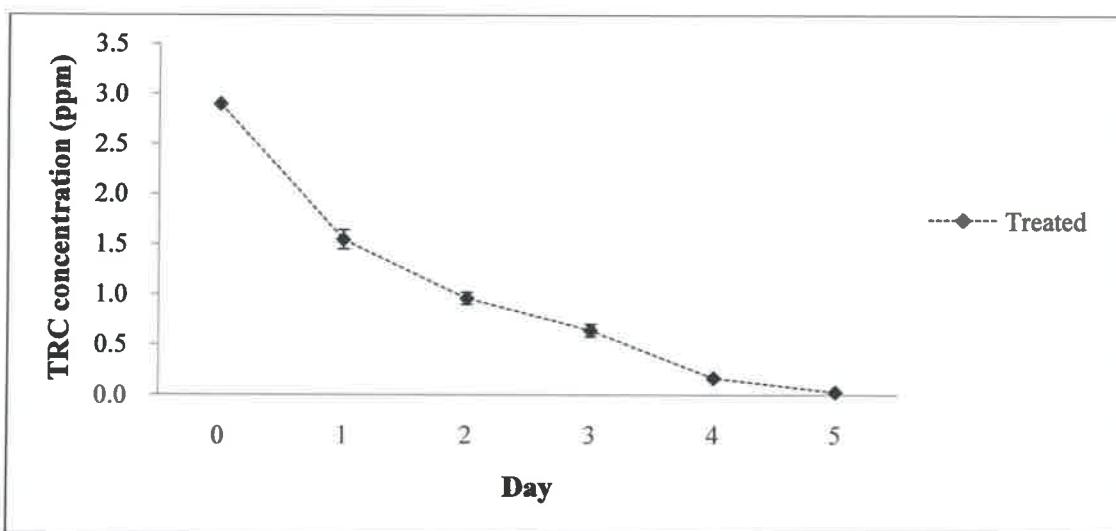


Figure 1. Change of TRC concentration of treated ballast seawater for 6 days

1.3 Influent water : Original water

1) $\geq 50 \mu\text{m}$ organismTable 4. Survival number of the organism larger than $50 \mu\text{m}$

| Sampling | Phyla/Divisions | Species | Survival number/ m^3 | Minimum Dimension (μm) |
|---------------|-----------------------------|--------------------------|---|-------------------------------------|
| Start | Heterokontophyta | <i>Coscinodiscus</i> sp. | 1870 ± 191 | 200 ~ 350 |
| | Arthropoda | <i>Oithona</i> sp. | 990 ± 330 | 130 ~ 180 |
| | | <i>Crustacean naupli</i> | 880 ± 381 | 170 ~ 210 |
| | | <i>Euclanus</i> sp. | 440 ± 504 | 260 ~ 400 |
| Middle | Heterokontophyta | <i>Coscinodiscus</i> sp. | 2333 ± 1137 | 200 ~ 350 |
| | Arthropoda | <i>Oithona</i> sp. | 1467 ± 115 | 130 ~ 180 |
| | | <i>Crustacean naupli</i> | 933 ± 462 | 170 ~ 210 |
| End | Heterokontophyta | <i>Coscinodiscus</i> sp. | 2803 ± 730 | 200 ~ 350 |
| | Arthropoda | <i>Oithona</i> sp. | 1353 ± 167 | 130 ~ 180 |
| | | <i>Crustacean naupli</i> | 870 ± 290 | 170 ~ 210 |
| | | <i>Euclanus</i> sp. | 387 ± 167 | 260 ~ 400 |
| Total | 4 species 2 phyla/divisions | | 4776 ± 618 | - |
| Acceptability | | | Influent condition: Acceptable ($\geq 10^2$ ind./ m^3) | - |

Data were presented as mean \pm S.D of three repeated measurement.
Some species were rare, the standard deviation was high.

2) 10 µm - 50 µm organism

Table 5. Survival number of the organism between 10 to 50 µm

| Sampling | Phyla/Divisions | Species | Survival number/mL (Mean) | Minimum Dimension (µm) |
|----------|-----------------------------|----------------------------|---|------------------------|
| Start | Heterokontophyta | <i>Thalassiosira</i> sp. | 403 ± 30 | 13 ~ 30 |
| | | <i>Chaetoceros</i> sp. | 202 ± 15 | 20 ~ 40 |
| | | <i>Skelctonema</i> sp. | 93 ± 9 | 13 ~ 19 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 60 ± 20 | 14 ~ 30 |
| | | <i>Ceratium</i> sp. | 15 ± 4 | 30 ~ 45 |
| Middle | Heterokontophyta | <i>Thalassiosira</i> sp. | 577 ± 42 | 13 ~ 30 |
| | | <i>Chaetoceros</i> sp. | 243 ± 51 | 20 ~ 40 |
| | | <i>Skelctonema</i> sp. | 72 ± 8 | 13 ~ 19 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 22 ± 17 | 15 ~ 25 |
| | Bacillariineae | <i>Pleurosigma</i> sp. | 10 ± 10 | 30 ~ 40 |
| End | Heterokontophyta | <i>Thalassiosira</i> sp. | 445 ± 42 | 13 ~ 30 |
| | | <i>Chaetoceros</i> sp. | 184 ± 11 | 20 ~ 40 |
| | | <i>Skelctonema</i> sp. | 114 ± 13 | 13 ~ 19 |
| | | <i>Melosira</i> sp. | 6 ± 2 | 18 ~ 30 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 33 ± 6 | 15 ~ 20 |
| Total | 7 species 3 phyla/divisions | | 827 ± 80 | - |
| | Acceptability | | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |

Data were presented as mean±S.D of three repeated measurement.
 Some species were rare, the standard deviation was high..

1.4 Elimination efficacy

1) $\geq 50 \mu\text{m}$ organism

Table 6. Survival number of the organism larger than $50 \mu\text{m}$

| Test substance | Sampling | Survival number/ m^3 | Acceptability | Elimination efficacy (%) |
|---|---------------|--|---|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 4180 \pm 1249 | Influent condition: Acceptable ($\geq 10^2 \text{ ind./m}^3$) | - |
| | | M ^{b)} 4733 \pm 1405 | | |
| | | E ^{c)} 5413 \pm 932 | | |
| Control (untreated) seawater | Day 5 (C5) | S 1773 \pm 428 | Effluent condition: Acceptable ($\geq 10 \text{ ind./m}^3$) | - |
| | | M 2040 \pm 416 | | |
| | | E 2333 \pm 202 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 \pm 0 | - | 100.0 ^{d)} |
| | | M 0 \pm 0 | | |
| | | E 0 \pm 0 | | |
| | Day 5 (T5) | S1 0 \pm 0 | Effluent condition: Acceptable (<10 ind./ m^3) | 100.0 ^{e)} |
| | | S2 0 \pm 0 | | |
| | | S3 0 \pm 0 | | |
| | | M1 0 \pm 0 | | |
| | | M2 0 \pm 0 | | |
| | | M3 0 \pm 0 | | |
| | | E1 0 \pm 0 | | |
| | | E2 0 \pm 0 | | |
| | | E3 0 \pm 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean \pm S.D of three repeated measurement.

2) 10 µm - 50 µm organism

Table 7. Survival number of the organism between 10 and 50 µm

| Test substance | Sampling | | Survival number/mL | Acceptability | Elimination efficacy (%) |
|------------------------------|------------|-----------------|--------------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 773 ± 40 | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |
| | | M ^{b)} | 925 ± 46 | | |
| | | E ^{c)} | 782 ± 21 | | |
| Control (untreated) seawater | Day 5 (C5) | S | 53 ± 13 | Effluent condition: Acceptable (≥ 10 ind./mL) | - |
| | | M | 83 ± 7 | | |
| | | E | 57 ± 3 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 5 (T5) | S1 | 0 ± 0 | Effluent condition: Acceptable (< 10 ind./mL) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

3) Heterotrophic bacteria**Table 8. Survival number of heterotrophic bacteria**

| Test substance | Sampling | | CFU / mL | Acceptability | Elimination efficacy (%) |
|------------------------------------|---------------|-----------------|------------|---------------|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 173 ± 31 | - | - |
| | | M ^{b)} | 513 ± 31 | | |
| | | E ^{c)} | 693 ± 210 | | |
| Control (untreated) seawater | Day 5 (C5) | S | 5777 ± 420 | - | - |
| | | M | 4043 ± 661 | | |
| | | E | 4377 ± 663 | | |
| Treated ballast seawater | Day 0 (T0) | S | 7 ± 6 | - | 98.8 ^{d)} |
| | | M | 7 ± 12 | | |
| | | E | 3 ± 6 | | |
| | Day 5 (T5) | S1 | 0 ± 0 | - | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

4) *Escherichia coli***Table 9. Survival number of *Escherichia coli***

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 21 ± 1 | - | - |
| | | M ^{b)} | 27 ± 1 | | |
| | | E ^{c)} | 25 ± 9 | | |
| Control (untreated) seawater | Day 5 (C5) | S | 17 ± 5 | - | - |
| | | M | 12 ± 4 | | |
| | | E | 19 ± 3 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 5 (T5) | S1 | 0 ± 0 | Effluent condition: Acceptable (<250 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

5) *Enterococcus faecalis*Table 10. Survival number of *Enterococcus faecalis*

| Test substance | Sampling | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|--------------------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 4 ± 0 | - | - |
| | | M ^{b)} 6 ± 2 | | |
| | | E ^{c)} 5 ± 2 | | |
| Control (untreated) seawater | Day 5 (C5) | S 0 ± 0 | - | - |
| | | M 0 ± 1 | | |
| | | E 0 ± 1 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 ± 0 | - | 100.0 ^{d)} |
| | | M 0 ± 0 | | |
| | | E 0 ± 0 | | |
| | Day 5 (T5) | S1 0 ± 0 | Effluent condition: Acceptable (<100 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 0 ± 0 | | |
| | | S3 0 ± 0 | | |
| | | M1 0 ± 0 | | |
| | | M2 0 ± 0 | | |
| | | M3 0 ± 0 | | |
| | | E1 0 ± 0 | | |
| | | E2 0 ± 0 | | |
| | | E3 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

6) *Vibrio cholera* O1, O139Table 11. Survival number of *Vibrio cholera* O1, O139

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 0 ± 0 | - | - |
| | | M ^{b)} | 0 ± 0 | | |
| | | E ^{c)} | 0 ± 0 | | |
| Control (untreated) seawater | Day 5 (C5) | S | 0 ± 0 | - | - |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | N.D ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 5 (T5) | S1 | 0 ± 0 | Effluent condition: Acceptable (<1 CFU/ 100ml) | N.D |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) N.D: Not detected

Data were presented as mean±S.D of three repeated measurement.

2. Validity

2.1 Water parameters measurement

- All measurements were tested more than three times.
- Water parameters were analyzed as soon as possible after sampling.

2.2 Biological efficacy test

- Influent condition in all tests was acceptable for IMO standards (MEPC58/2/4).
 - The organism larger than 50 µm: $\geq 10^2$ individuals/m³
 - The organism between 10 µm and 50 µm: $\geq 10^2$ individuals / mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| OwS | 4180 ± 1249 | 773 ± 40 |
| OwM | 4733 ± 1405 | 925 ± 46 |
| OwE | 5413 ± 932 | 782 ± 21 |

- Discharge results from the control water were a concentration more than the values in regulation D2.1.
 - The organism larger than 50 µm: ≥ 10 individuals/m³
 - The organism between 10 µm and 50 µm: ≥ 10 individuals/mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| C5S | 1773 ± 428 | 53 ± 13 |
| C5M | 2040 ± 416 | 83 ± 7 |
| C5E | 2333 ± 202 | 57 ± 3 |

- The samples should be analyzed as soon as possible after sampling.

| Date | Sample | Sampling time | Test ending time |
|-------|-------------|---------------|------------------|
| Day 0 | OwS | 10:50 | 18:30 |
| | OwM | 10:58 | 18:30 |
| | OwE | 11:00 | 18:30 |
| | T0S | 10:50 | 18:30 |
| | T0M | 10:58 | 18:30 |
| | T0E | 11:10 | 18:30 |
| Day 5 | C5S | 13:30 | 21:00 |
| | C5M | 13:42 | 21:00 |
| | C5E | 13:55 | 21:00 |
| | T5/S1, 2, 3 | 14:15 | 21:00 |
| | T5/M1, 2, 3 | 14:28 | 21:00 |
| | T5/E1, 2, 3 | 14:39 | 21:00 |

3. Conclusion

3.1 Biological efficacy in treated ballast seawater by Purimar™ BWMS

- Treated ballast seawater by Purimar™ BWMS was capable of eliminating organism larger than 50 µm with an efficiency of 100%.
- Treated ballast seawater water by Purimar™ BWMS was capable of eliminating organism between 10 and 50 µm with an efficiency of 100 %.
- Treated ballast seawater by Purimar™ BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by Purimar™ BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by Purimar™ BWMS was capable of eliminating *Escherichia coli* with an efficiency of 100 %.
- Treated ballast seawater by Purimar™ BWMS was capable of eliminating *Enterococcus faecalis* with an efficiency of 100 %.
- *Vibrio cholera* O1, O139 were not detected.
- **Therefore, treated ballast seawater by Purimar™ BWMS was capable of removing zooplankton, phytoplankton, bacteria. And Purimar™ BWMS showed discharge of treated ballast water in compliance with regulation D-2.**

4. Appendix

Appendix 1. Water parameters at field

| Date | Sample ID | R ^{a)} | pH | Temp (°C) | Salinity (%) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------------|------|-----------|--------------|-----------|-----------------|--------------|
| Day 0 | OwS | R1 | 7.09 | 12.26 | 22.00 | 7.96 | 3.0 | 0.602 |
| | | R2 | 7.10 | 12.25 | 22.00 | 7.96 | 2.9 | 0.602 |
| | | R3 | 7.10 | 12.25 | 22.01 | 7.96 | 2.9 | 0.602 |
| | OwM | R1 | 7.21 | 11.53 | 22.11 | 7.82 | 2.7 | 0.652 |
| | | R2 | 7.22 | 11.53 | 22.12 | 7.81 | 2.7 | 0.652 |
| | | R3 | 7.22 | 11.52 | 22.13 | 7.81 | 2.6 | 0.652 |
| | OwE | R1 | 7.36 | 11.36 | 21.78 | 7.78 | 2.5 | 0.631 |
| | | R2 | 7.36 | 11.36 | 21.78 | 7.78 | 2.5 | 0.631 |
| | | R3 | 7.36 | 11.36 | 21.78 | 7.78 | 2.5 | 0.631 |
| | T0S | R1 | 7.34 | 12.01 | 22.46 | 7.43 | 1.2 | 0.000 |
| | | R2 | 7.35 | 12.01 | 22.46 | 7.42 | 1.1 | 0.000 |
| | | R3 | 7.35 | 12.02 | 22.46 | 7.41 | 1.1 | 0.000 |
| | T0M | R1 | 7.45 | 12.00 | 21.72 | 7.56 | 1.0 | 0.000 |
| | | R2 | 7.45 | 11.98 | 21.72 | 7.56 | 1.0 | 0.000 |
| | | R3 | 7.45 | 11.98 | 21.72 | 7.56 | 1.0 | 0.000 |
| | T0E | R1 | 7.51 | 11.96 | 22.12 | 7.34 | 1.3 | 0.000 |
| | | R2 | 7.51 | 11.96 | 22.13 | 7.36 | 1.3 | 0.000 |
| | | R3 | 7.51 | 11.96 | 22.13 | 7.36 | 1.2 | 0.000 |

a) R: Replicate

Test Report**SET-10-004**

| Date | Sample ID & lot No. | R ^{a)} | pH | Temp (°C) | Salinity (%) | DO (mg/L) | Turbidity (NTU) | Chlorophyla |
|-------|---------------------|-----------------|------|-----------|--------------|-----------|-----------------|-------------|
| Day 5 | C5S | R1 | 7.19 | 26.44 | 23.70 | 7.84 | 3.2 | 0.106 |
| | | R2 | 7.19 | 26.45 | 23.70 | 7.84 | 3.2 | 0.106 |
| | | R3 | 7.20 | 26.45 | 23.70 | 7.84 | 3.1 | 0.106 |
| | C5M | R1 | 7.24 | 25.90 | 23.66 | 7.47 | 3.1 | 0.102 |
| | | R2 | 7.24 | 25.90 | 23.67 | 7.47 | 3.1 | 0.102 |
| | | R3 | 7.24 | 25.90 | 23.67 | 7.47 | 3.1 | 0.102 |
| | C5E | R1 | 7.38 | 25.86 | 23.66 | 7.43 | 2.9 | 0.105 |
| | | R2 | 7.39 | 25.86 | 23.65 | 7.43 | 3.0 | 0.105 |
| | | R3 | 7.39 | 25.85 | 23.67 | 7.43 | 3.0 | 0.105 |
| | T5/S1 | R1 | 7.73 | 25.23 | 23.09 | 7.03 | 1.4 | 0.000 |
| | | R2 | 7.73 | 25.23 | 23.08 | 7.03 | 1.4 | 0.000 |
| | | R3 | 7.73 | 25.23 | 23.08 | 7.03 | 1.4 | 0.000 |
| | T5/S2 | R1 | 7.79 | 25.00 | 23.11 | 6.82 | 1.5 | 0.000 |
| | | R2 | 7.79 | 24.99 | 23.11 | 6.82 | 1.5 | 0.000 |
| | | R3 | 7.80 | 24.99 | 23.12 | 6.82 | 1.5 | 0.000 |
| | T5/S3 | R1 | 7.93 | 25.11 | 23.10 | 6.95 | 1.8 | 0.000 |
| | | R2 | 7.93 | 25.11 | 23.11 | 6.95 | 1.8 | 0.000 |
| | | R3 | 7.93 | 25.12 | 23.11 | 6.95 | 1.8 | 0.000 |
| | T5/M1 | R1 | 7.93 | 25.12 | 23.11 | 6.96 | 1.8 | 0.000 |
| | | R2 | 7.93 | 25.12 | 23.11 | 6.96 | 1.8 | 0.000 |
| | | R3 | 7.93 | 25.12 | 23.11 | 6.96 | 1.8 | 0.000 |
| | T5/M2 | R1 | 7.96 | 24.95 | 23.10 | 6.85 | 1.1 | 0.000 |
| | | R2 | 7.96 | 24.95 | 23.10 | 6.85 | 1.1 | 0.000 |
| | | R3 | 7.96 | 24.95 | 23.10 | 6.85 | 1.1 | 0.000 |
| | T5/M3 | R1 | 7.98 | 25.06 | 23.10 | 6.91 | 1.5 | 0.000 |
| | | R2 | 7.98 | 25.07 | 23.10 | 6.91 | 1.5 | 0.000 |
| | | R3 | 7.98 | 25.07 | 23.10 | 6.91 | 1.5 | 0.000 |
| | T5/E1 | R1 | 8.01 | 25.23 | 23.12 | 7.03 | 1.6 | 0.000 |
| | | R2 | 8.01 | 25.23 | 23.12 | 7.03 | 1.6 | 0.000 |
| | | R3 | 8.01 | 25.23 | 23.12 | 7.03 | 1.7 | 0.000 |
| | T5/E2 | R1 | 8.01 | 25.15 | 23.16 | 6.98 | 0.9 | 0.000 |
| | | R2 | 8.01 | 25.16 | 23.16 | 6.98 | 1.1 | 0.000 |
| | | R3 | 8.02 | 25.16 | 23.16 | 6.98 | 1.0 | 0.000 |
| | T5/E3 | R1 | 8.02 | 25.32 | 23.16 | 7.09 | 1.5 | 0.000 |
| | | R2 | 8.02 | 25.32 | 23.16 | 7.09 | 1.6 | 0.000 |
| | | R3 | 8.02 | 25.32 | 23.16 | 7.09 | 1.4 | 0.000 |

Appendix 2. Water parameters (TOC, DOC, TSS) of original water (Ow)

| Date | Sample ID | R ^{a)} | TOC ^{b)} (mg/L) | DOC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | R1 | 2.79 | 1.73 | 7.2 |
| | | R2 | 2.77 | 1.70 | 6.6 |
| | | R3 | 2.73 | 1.66 | 6.4 |
| | OwM | R1 | 2.80 | 1.71 | 7.6 |
| | | R2 | 2.80 | 1.68 | 6.6 |
| | | R3 | 2.78 | 1.69 | 7.0 |
| | OwE | R1 | 2.80 | 1.72 | 6.4 |
| | | R2 | 2.78 | 1.70 | 7.0 |
| | | R3 | 2.77 | 1.74 | 6.8 |

a) R: Replicate

b) TOC: Total Organic Carbon (TOC=DOC+POC)

c) DOC: Dissolved Organic Carbon

d) TSS: Total Suspended Solids

Appendix 3. TRC data

| Date | Group | TRC concentration (ppm) | | | Mean | S.D. ^b |
|--------------|---------|-------------------------|------|------|------|-------------------|
| Day 0 | Control | 0.06 | 0.07 | 0.05 | 0.06 | 0.01 |
| | Treated | 2.90 | 2.89 | 2.91 | 2.90 | 0.01 |
| Day 1 | Control | 0.03 | 0.04 | 0.05 | 0.04 | 0.01 |
| | Treated | 1.65 | 1.53 | 1.46 | 1.55 | 0.10 |
| Day 2 | Control | 0.04 | 0.04 | 0.03 | 0.04 | 0.01 |
| | Treated | 1.02 | 0.90 | 0.96 | 0.96 | 0.06 |
| Day 3 | Control | 0.03 | 0.02 | 0.03 | 0.03 | 0.01 |
| | Treated | 0.71 | 0.59 | 0.62 | 0.64 | 0.06 |
| Day 4 | Control | 0.03 | 0.04 | 0.03 | 0.03 | 0.01 |
| | Treated | 0.19 | 0.16 | 0.15 | 0.17 | 0.02 |
| Day 5 | Control | 0.02 | 0.03 | 0.02 | 0.02 | 0.01 |
| | Treated | 0.04 | 0.02 | 0.02 | 0.03 | 0.01 |

^a S.D: standard deviation^{*} N.D: Not detected

Appendix 4. Survival number of $\geq 50\mu\text{m}$ organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|---------------------------|-------------------------------|------|------|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Coscinodiscus</i> sp. | 1980 | 1650 | 1980 | 1870 | 191 |
| | | <i>Oithona</i> sp. | 660 | 990 | 1320 | 990 | 330 |
| | | <i>Crustacean nauplii</i> | 660 | 660 | 1320 | 880 | 381 |
| | | <i>Euclanus</i> sp. | 330 | 0 | 990 | 440 | 504 |
| | OwM | <i>Coscinodiscus</i> sp. | 2000 | 1400 | 3600 | 2333 | 1137 |
| | | <i>Oithona</i> sp. | 1400 | 1600 | 1400 | 1467 | 115 |
| | | <i>Crustacean nauplii</i> | 1200 | 400 | 1200 | 933 | 462 |
| | OwE | <i>Coscinodiscus</i> sp. | 2900 | 2030 | 3480 | 2803 | 730 |
| | | <i>Oithona</i> sp. | 1450 | 1160 | 1450 | 1353 | 167 |
| | | <i>Crustacean nauplii</i> | 1160 | 580 | 870 | 870 | 290 |
| | | <i>Euclanus</i> sp. | 290 | 580 | 290 | 387 | 167 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 5. Survival number of $\geq 50\mu\text{m}$ organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|---------------------------|-------------------------------|------|------|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 5 | C5S | <i>Coscinodiscus</i> sp. | 840 | 1120 | 1400 | 1120 | 280 |
| | | <i>Oithona</i> sp. | 560 | 280 | 560 | 467 | 162 |
| | | <i>Crustacean nauplii</i> | 0 | 280 | 280 | 187 | 162 |
| | C5M | <i>Coscinodiscus</i> sp. | 1080 | 1080 | 720 | 960 | 208 |
| | | <i>Oithona</i> sp. | 1440 | 720 | 1080 | 1080 | 360 |
| | C5E | <i>Coscinodiscus</i> sp. | 1400 | 700 | 1050 | 1050 | 350 |
| | | <i>Oithona</i> sp. | 700 | 1750 | 1400 | 1283 | 535 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 6. Survival number of $\geq 50\mu\text{m}$ organism in treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|----------------|-------------------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | TOS | - | 0 | 0 | 0 | 0 | 0 |
| | TOM | - | 0 | 0 | 0 | 0 | 0 |
| | TOE | - | 0 | 0 | 0 | 0 | 0 |
| Day 5 | T5/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 7. Survival number of 10 μm - 50 μm organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|-------|-----------|----------------------------|----------------------|-----|-----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Thalassiosira</i> sp. | 372 | 431 | 407 | 403 | 30 |
| | | <i>Chaetoceros</i> sp. | 205 | 186 | 215 | 202 | 15 |
| | | <i>Skeletonema</i> sp. | 93 | 84 | 102 | 93 | 9 |
| | | <i>Protoperidinium</i> sp. | 45 | 83 | 51 | 60 | 20 |
| | | <i>Ceratium</i> sp. | 13 | 20 | 13 | 15 | 4 |
| Day 0 | OwM | <i>Thalassiosira</i> sp. | 612 | 531 | 589 | 577 | 42 |
| | | <i>Chaetoceros</i> sp. | 188 | 254 | 288 | 243 | 51 |
| | | <i>Skeletonema</i> sp. | 71 | 80 | 65 | 72 | 8 |
| | | <i>Protoperidinium</i> sp. | 40 | 6 | 21 | 22 | 17 |
| | | <i>Pleurosigma</i> sp. | 21 | 5 | 4 | 10 | 10 |
| Day 0 | OwE | <i>Thalassiosira</i> sp. | 475 | 463 | 397 | 445 | 42 |
| | | <i>Chaetoceros</i> sp. | 172 | 186 | 194 | 184 | 11 |
| | | <i>Skeletonema</i> sp. | 100 | 117 | 126 | 114 | 13 |
| | | <i>Melosira</i> sp. | 7 | 7 | 3 | 6 | 2 |
| | | <i>Protoperidinium</i> sp. | 31 | 29 | 40 | 33 | 6 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 8. Survival number of 10 μm - 50 μm organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|--------------|-----------|----------------------------|----------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 5 | C5S | <i>Thalassiosira</i> sp. | 32 | 49 | 36 | 39 | 9 |
| | | <i>Chaetoceros</i> sp. | 7 | 12 | 11 | 10 | 3 |
| | | <i>Ceratium</i> sp. | 3 | 6 | 4 | 4 | 2 |
| | C5M | <i>Thalassiosira</i> sp. | 52 | 51 | 62 | 55 | 6 |
| | | <i>Chaetoceros</i> sp. | 29 | 20 | 25 | 25 | 5 |
| | | <i>Protoperidinium</i> sp. | 2 | 5 | 2 | 3 | 2 |
| | C5E | <i>Thalassiosira</i> sp. | 39 | 44 | 41 | 41 | 3 |
| | | <i>Chaetoceros</i> sp. | 16 | 10 | 12 | 13 | 3 |
| | | <i>Melosira</i> sp. | 2 | 6 | 2 | 3 | 2 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

**Appendix 9. Survival number of 10 μm - 50 μm organism in treated ballast seawater
by treatment of PurimarTM BWMS**

| Date | Sample ID | Classification | Survival number/mL | | | Mean | SD ^{b)} |
|-------|-----------|----------------|--------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | TOS | - | 0 | 0 | 0 | 0 | 0 |
| | TOM | - | 0 | 0 | 0 | 0 | 0 |
| | TOE | - | 0 | 0 | 0 | 0 | 0 |
| Day 5 | T5/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T5/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 10. Number of heterotrophic bacteria in original water, control (untreated) seawater and treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | CFU/mL | | | Mean | SD ^{b)} | |
|-------|-----------|------------------|-------|-------|-------|------------------|-----|
| | | R1 ^{a)} | R2 | R3 | | | |
| Day 0 | Ow | Start | 140 | 180 | 200 | 173 | 31 |
| | | Middle | 480 | 520 | 540 | 513 | 31 |
| | | End | 480 | 700 | 900 | 693 | 210 |
| | T0 | Start | 0 | 10 | 10 | 7 | 6 |
| | | Middle | 0 | 0 | 20 | 7 | 12 |
| | | End | 0 | 0 | 10 | 3 | 6 |
| Day 5 | C5 | S | 5,420 | 5,670 | 6,240 | 5,777 | 420 |
| | | M | 3,440 | 3,940 | 4,750 | 4,043 | 661 |
| | | E | 3,820 | 4,200 | 5,110 | 4,377 | 663 |
| | T5 | S1 | 0 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 11. Number of *Escherichia coli* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} | |
|--------------|-----------|------------------|----|----|------|------------------|----|
| | | R1 ^{a)} | R2 | R3 | | | |
| Day 0 | Ow | Start | 21 | 22 | 21 | 1 | 21 |
| | | Middle | 27 | 28 | 27 | 1 | 27 |
| | | End | 27 | 33 | 25 | 9 | 27 |
| | T0 | Start | 0 | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 | 0 |
| Day 5 | C5 | S | 15 | 22 | 19 | 5 | 15 |
| | | M | 13 | 15 | 14 | 1 | 13 |
| | | E | 19 | 22 | 21 | 2 | 19 |
| | T5 | S1 | 0 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 12. Number of *Enterococcus faecalis* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 4 | 4 | 4 | 4 |
| | | Middle | 5 | 6 | 8 | 6 |
| | | End | 4 | 5 | 7 | 5 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day 5 | C5 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 1 | 0 |
| | | E | 0 | 0 | 1 | 0 |
| | T5 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

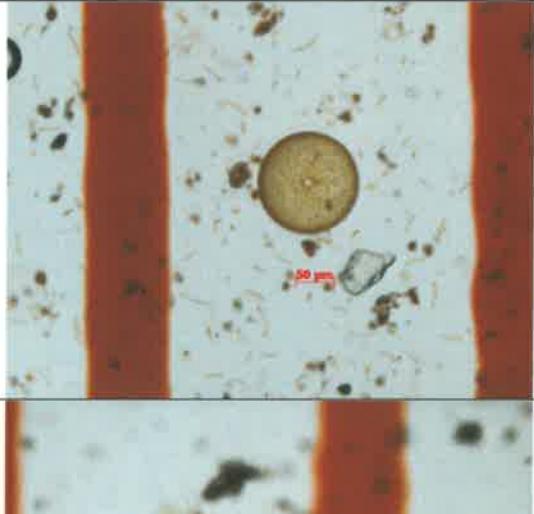
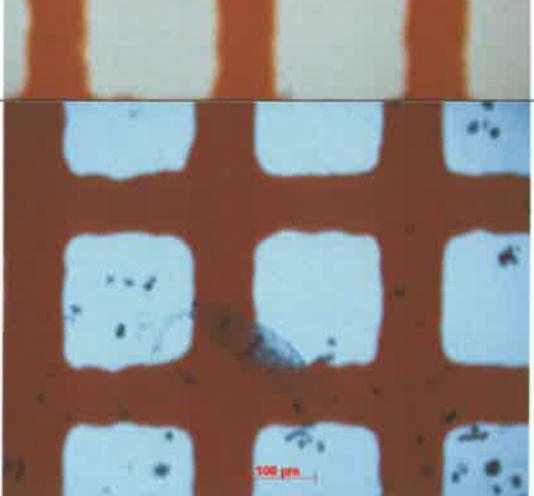
Appendix 13. Number of *Vibrio cholera* O1, O139 in original water, control (untreated) seawater and treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day 5 | C5 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 0 | 0 |
| | | E | 0 | 0 | 0 | 0 |
| | T5 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

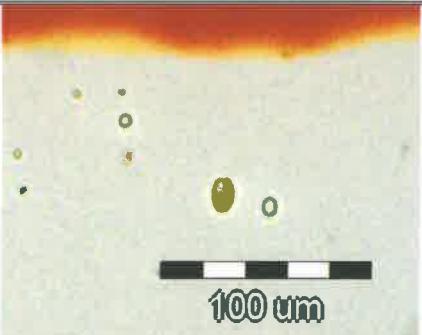
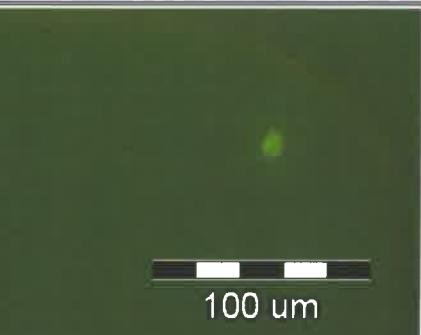
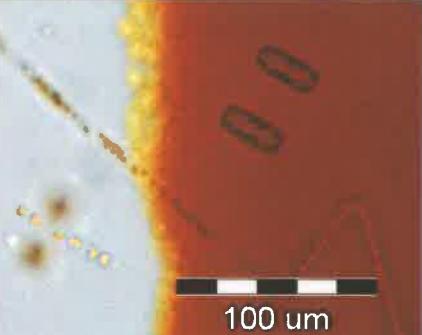
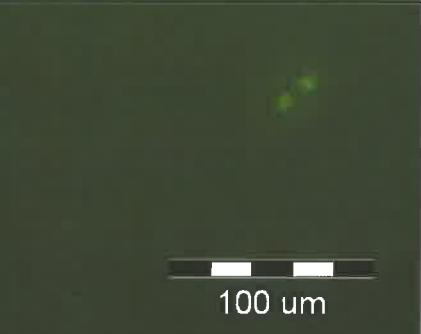
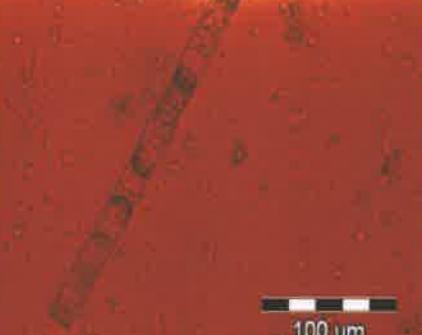
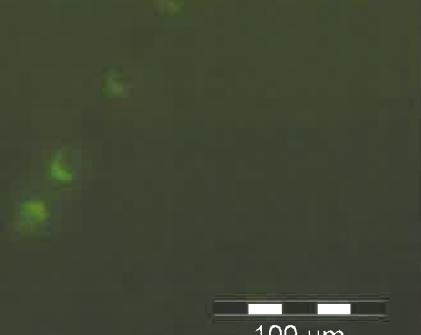
Appendix 14. The microscope image of $\geq 50\mu\text{m}$ in original water (Day0)

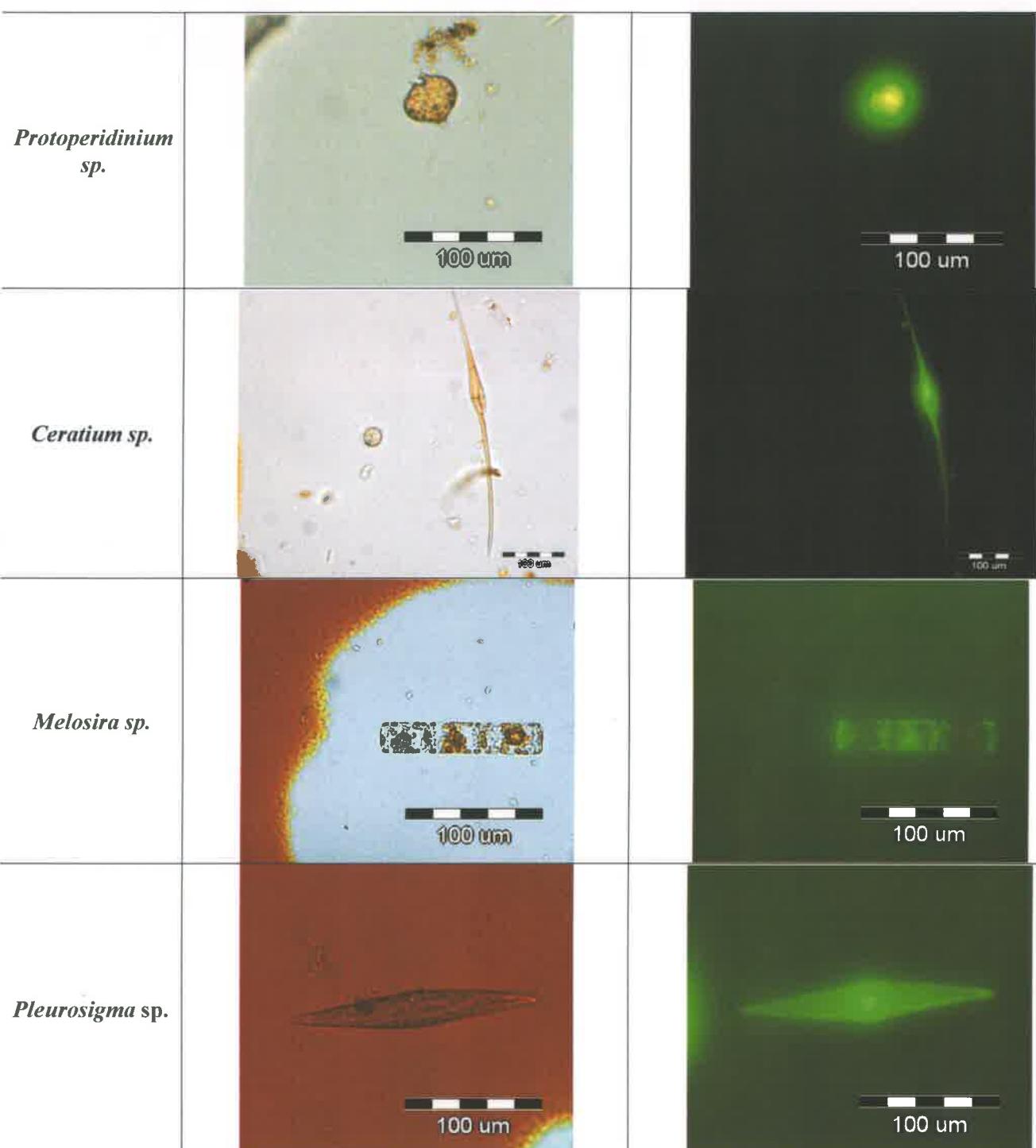
| Specific Name | Original water |
|---------------------------|--|
| <i>Coscinodiscus</i> sp. |  |
| <i>Oithona</i> sp. |  |
| <i>Crustacean nauplii</i> |  |
| <i>Euclanus</i> sp. |  |

Appendix 15. The microscope image of $\geq 50\mu\text{m}$ in treated ballast seawater by treatment of PurimarTM BWMS (Day0)

| Specific Name | Control (untreated) seawater |
|---------------|---|
| |  A high-magnification microscope image showing a dense, granular cluster of microorganisms. The cluster is roughly circular and light-colored against a dark, reddish-brown background. The individual organisms appear as small, dark, irregular shapes and filaments. |

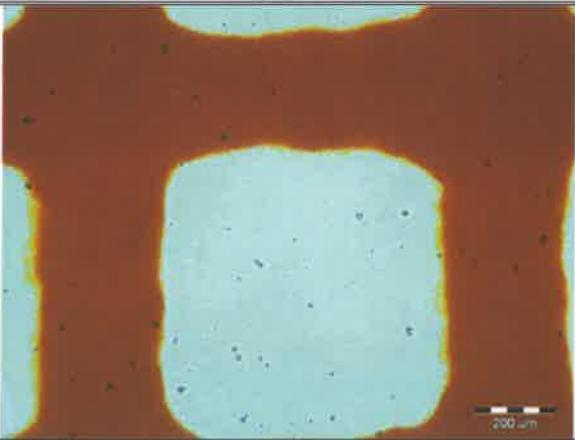
Appendix 16. The microscope image of $10\mu\text{m}$ - $50\mu\text{m}$ in original water (Day0)

| Specific Name | Original water | |
|----------------------------|---|---|
| | Optical | Fluorescence |
| <i>Tetraselmis suecica</i> |  |  |
| <i>Thalassiosira sp.</i> |  |  |
| <i>Chaetoceros sp.</i> |  |  |
| <i>Skeletonema sp.</i> |  |  |



Magnification : 200

Appendix 17. The microscope image of 10 μm –50 μm in treated ballast seawater by treatment of PurimarTM BWMS (Day0)

| Specific Name | Treated ballast seawater | |
|---------------|---|--|
| | Optical | Fluorescence |
| - |  |  |

Magnification : 200

Appendix 18. The image of heterotrophic bacteria: Day 0**Treated ballast seawater****Start****Middle****End****Original water****Start****Middle****End**

Appendix 19. The image of heterotrophic bacteria: Day 5

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



Middle(2)



Middle(3)



End(1)



End(2)

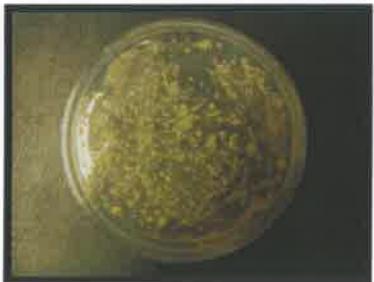


End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 20. The image of *Escherichia coli*: Day 0

Treated ballast seawater

Start

Middle

End

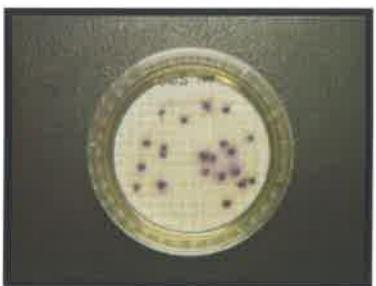


Original water

Start

Middle

End



Appendix 21. The image of *Escherichia coli*: Day 5

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



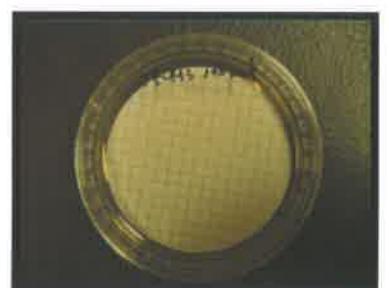
Middle(1)



Middle(2)



Middle(3)



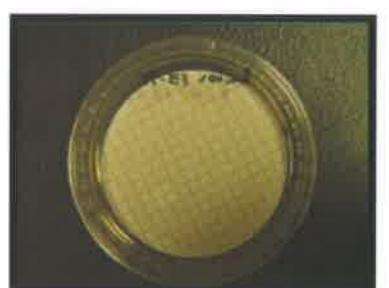
End(1)



End(2)



End(3)



Control (untreated) seawater

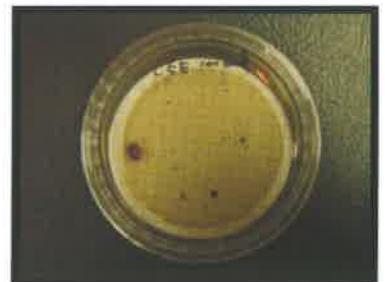
Start



Middle



End



Appendix 22. The image of *Enterococcus faecalis*: Day 0

Treated ballast seawater

Start



Middle



End



Original water

Start



Middle



End



Appendix 23. The image of *Enterococcus faecalis*: Day 5

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 24. The image of *Vibrio cholera* O1, O139: Day 0

Original water

Start



Middle



Appendix 25. The image of *Vibrio cholera* O1, O139: Day 5

Control (untreated) seawater

Start

Middle

End



5. Attachment

- 5.1 Loading record
- 5.2 Sampling check & custody sheet
- 5.3 Freezing keeping sheet
- 5.4 Chain of custody record
- 5.5 Sample receipt form
- 5.6 Water parameter measurement sheet (I)
- 5.7 TOC measurement sheet
- 5.8 TSS(Total suspended solid) measurement sheet
- 5.9 TRC measurement sheet(For field)
- 5.10 Microbiology test sheet
- 5.11 Test results sheet with microbiology
- 5.12 Vibrio cholera O1, O139
- 5.13 Test results sheet – Counting
- 5.14 Test results sheet – Classification(II)

첨부(2)

시험번호 : SET-10-005

시험항구 : Kaoshiung(Taiwan)

Test Plan

| | |
|--------------|--|
| Project name | Purimar™ |
| Study number | TW-Ship(5) |
| Test number | SET-10-005 |
| Port | Kaoshiung (Taiwan) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | JaeYoung Back  <small>(Sign)</small> |

1. Test title

Efficacy test of Purimar™ Ballast Water Management System (shipboard scale)

2. Test purpose

- 2.1 The objective of the present test is to evaluate the efficacy of Purimar™ Ballast Water Management System(BWMS).
- 2.2 We will determine elimination efficacy of organism lager than 50 µm, organism between 10 and 50 µm and bacteria by treatment of Purimar™ BWMS.

3. Schedule

3.1 The expecting port of shipboard test : Kaoshiung(Taiwan)

3.2 Equipment usage(treatment) schedule

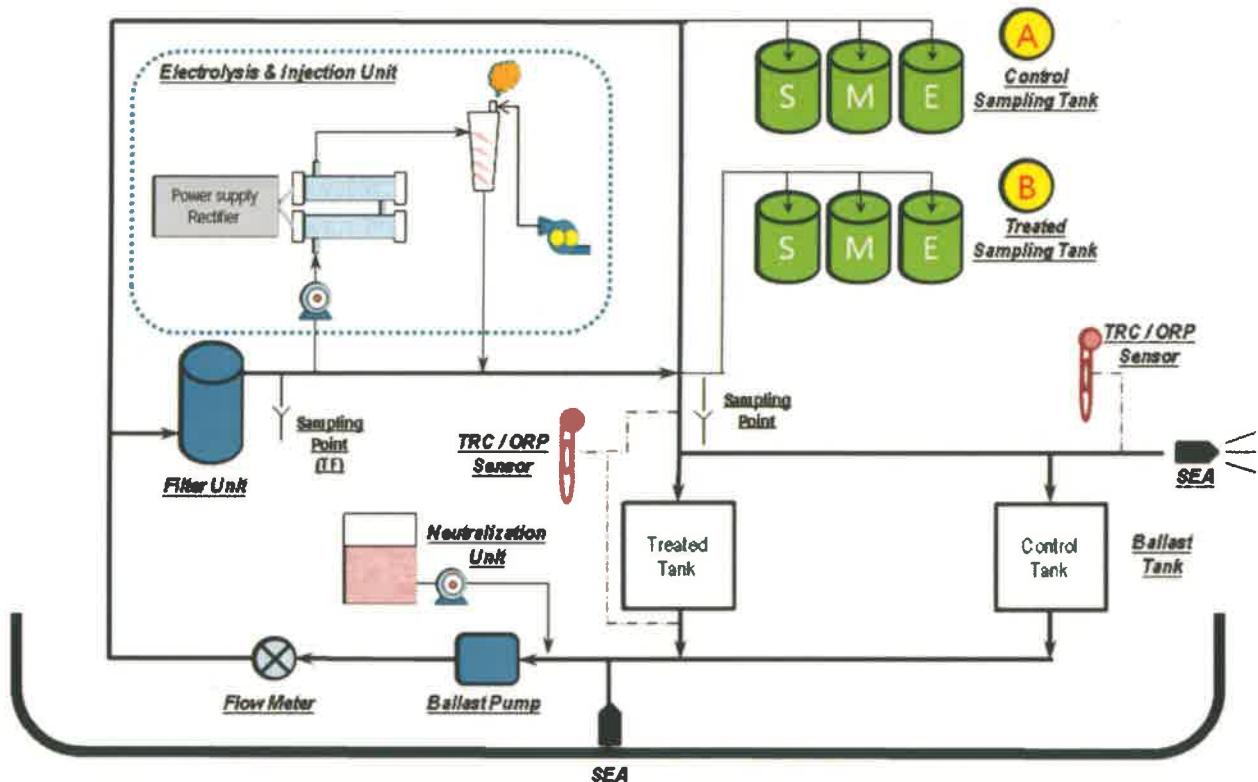
| Test time | Date | |
|-----------|--------------------|--------------|
| | Purimar™ Treatment | Deballasting |
| Day 0 | Dec 18, 2010 | - |
| Day 7 | - | Dec 25, 2010 |

3.3 Test schedule

| Test time | Date | | | |
|-----------|--|-----------------------------------|-----------------------------------|--------------------------------------|
| | *Water parameters * $\geq 50 \mu\text{m}$ Organisms *10~50 μm Organisms | *Coliform *Enterococcus group | * <i>V. cholera</i> | * Heterotrophic bacteria (Marine) |
| Day 0 | Dec 18, 2010 | Dec 18, 2010 ~ Dec 19, 2010 | Dec 18, 2010 ~ Dec 20, 2010 | Dec 18, 2010 ~ Dec 21, 2010 |
| Day 7 | Dec 25, 2010 | Dec 25, 2010 ~ Dec 26, 2010 | Dec 25, 2010 ~ Dec 27, 2010 | Dec 25, 2010 ~ Dec 28, 2010 |

4. Sampling procedures

4.1 Sampling point



| Sampling point | Day | Sample name | Parameters |
|----------------|-------|---|--|
| A | Day 0 | Original water / Control (untreated) seawater | Water parameters ^{a)} Organism ^{b)} Bacteria ^{c)} |
| B | Day 7 | Treated ballast seawater | Water parameters ^{d)} Organism ^{b)} Bacteria ^{c)} |

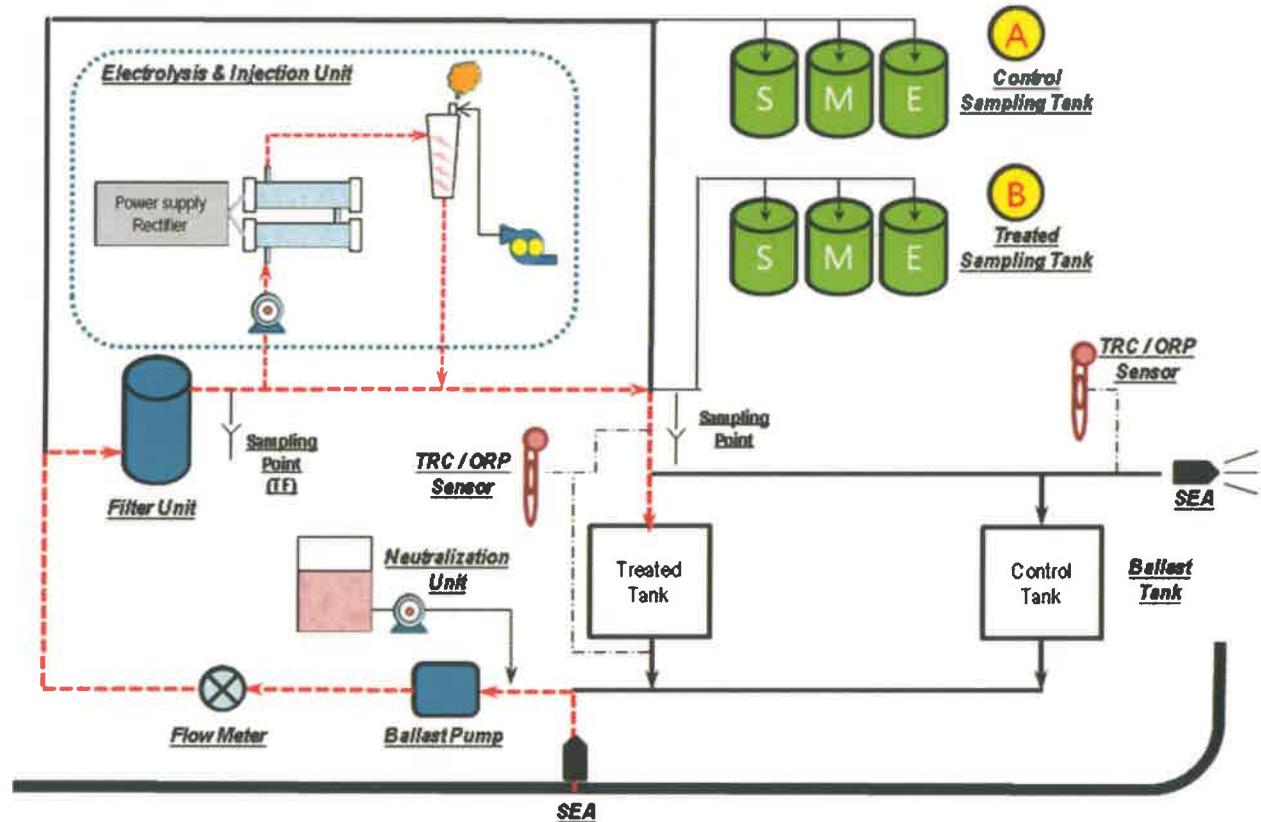
a) Water parameters: pH, Temperature, Salinity, DO, Turbidity, DOC, POC, TSS

b) Organism: $\geq 50 \mu\text{m}$ Organisms, $10\mu\text{m} - 50\mu\text{m}$ Organisms

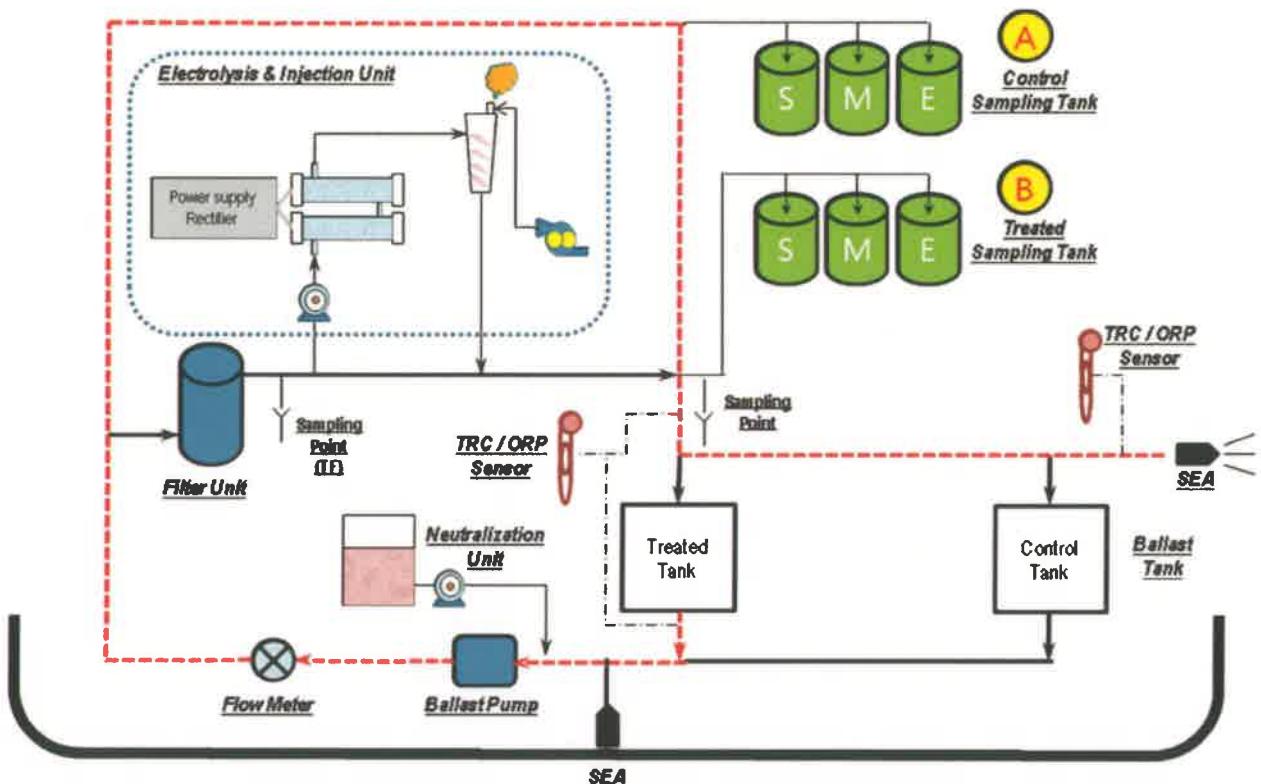
c) Bacteria: Heterotrophic bacteria, Coliform, Enterococcus group, *V. cholera*

d) Water parameters: pH, Temperature, Salinity, DO, Turbidity

4.2 Ballasting mode



4.3 Deballasting mode



4.4 Sampling procedures

- Collection and handling of field samples from the HANJIN “the DURBAN” will be undertaken by a team from MBDC and NLP Co., Ltd, using standard water sample collection methods and in accordance with the G8 Guidelines. Standard operating procedure (SOP) - SOP-BWMS-023 is employed to provide consistency and reproducibility to the sampling methods used by field personnel.
- Water samples will be taken from both the control (untreated) and treated tanks at two times intervals following treatment – at the ballasting (immediately after treatment, day 0), and at the discharge (deballasting or neutralizing agent treatment after eight days, day 7), and identified as numbered sampling points.
- All sampling equipments, apparatus and containers are prepared in accordance with EPA’s Coastal 2000 Field Operation Manual. They are packed into exclusive cases.
- Water samples should be directly taken full up in a sample bottles after washing by sample water. When sample bottles are pre-preserved, the bottles should not be rinsed but be filled once with sample.
- Water parameters of samples are analyzed as soon as possible after collection at a field. The collected samples are transported to the laboratory in the DURBAN for the analysis.
- When the samples are arrived to laboratory, laboratory personnel receive the samples and entered the samples into the laboratory. The laboratory custodian will open the sample and carefully check the contents for evidence of leakage
- Sample handling will be performed so as to collect, store, submit to the laboratory and analyze representative samples using methods as specified in the test plans.

4.5 Test substances of Day 0

- Influent water (Original water)

| Parameter | Sampling point | Sample ID & Lot No. |
|------------------|----------------|---------------------|
| Organisms | A | OwS-1218 |
| Bacteria | | OwM-1218 |
| Water parameters | | OwE-1218 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T0S-1218 |
| Heterotrophic bacteria | | T0M-1218 |
| Water parameters | | T0E-1218 |

4.6 Test substances of Day 7

- Control (untreated) seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------|---------------|---------------------|
| Organisms | A | C7S-1225 |
| Bacteria | | C7M-1225 |
| Water parameters | | C7E-1225 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T7/S1, 2, 3-1225 |
| Heterotrophic bacteria | | T7/M1, 2, 3-1225 |
| Water parameters | | T7/E1, 2, 3-1225 |

5. Test procedures

5.1 Test design

| | |
|--------------------------|--|
| Test system | Purimar™ (shipboard scale) |
| Test substance | Original water: 100% Control (untreated) seawater: 100% Treated ballast seawater: 100% |
| Dilution water | Filtered natural seawater (FNS) |
| TRC concentration | Control: 0.0 ppm Treated: 3.0 ppm(\pm 0.5 ppm) |
| Sampling time | Day 0, 7 |

5.2 Test method

5.2.1 Water parameters measurement

- 1) Water parameters (temperature, pH, DO, salinity, turbidity) of samples at ship(HANJIN DURBAN) are measured using an MS5 according to SOP-BWMS-022.
- 2) Water parameters (DOC, POC) of samples at MBDC lab. are measured using vario TOC cube according to SOP-BWMS-021.
- 3) Water parameter (TSS) of samples at MBDC lab. is measured according to SOP-BWMS-005.

5.2.2 Biological efficacy test

- 1) $\geq 50\mu\text{m}$ organism
 - ① Concentration
 - Sample can be concentrated with 32 μm sieve.
 - After concentration, wash with filtered natural seawater to gather organisms.
 - Concentrated sample transfer into glass beaker and fill up to 100 mL with filtered natural seawater.
 - ② Analysis
 - General method
 - Analysis under stereo microscope with dark field (alive: movement /dead: lack of movement).
 - 1 ~ 20 mL of concentration sample place on a counting chamber (sedgewick-Rafter cell or Bogorov counting chamber).
 - The number of observations must be more than three.

- Analysis by process of dyeing
 - Making of FDA Stock solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
 - Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
 - Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
 - 1 mL of dyed sample place on a counting chamber.
 - Waiting for 10 minutes for cell staining.
 - Turn on mercury burner of microscope and apply to a fluorescent filter.
 - Viable cells represent green color.
 - Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

2) 10 μ m - 50 μ m organism

① General method

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- 0.1 mL of fixed sample place on a counting chamber (sedgewick-Rafter cell).
- Waiting for 5 minutes for cell sinking.
- The number of observations must be more than three.

② Staining

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- Making of FDA Stock Solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
- Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
- Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
- Dyed sample place on a counting chamber.
- Waiting for 10 minutes for cell staining.
- Turn on mercury burner of microscope and apply to a fluorescent filter.
- Viable cells represent green color.
- Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

3) Heterotrophic bacteria

- Standard: APHA 9215 (Heterotrophic plate count: 2005)
- SOP: SOP-BWTS-009
- Medium: Marine agar (DIFCO, Cat No 212185, Lot No 8129045)
- Method: Sample smear on the agar plate.
- Sample volume: 0.1 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 25°C, 3 days.
- Data analysis: Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates promptly after incubation. And calculate count per 1 mL.

4) *Escherichia coli*

- Standard: EPA 1603 [*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane - Thermo tolerant *Escherichia coli* Agar (Modified mTEC) : 2006]
- SOP: SOP-BWTS-010
- Medium: mTEC agar (DIFCO, Cat No 214880, Lot No 8171842)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Escherichia coli* (ATCC No #11775)
- Negative control: *Enterococcus faecalis* (ATCC No #19433)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: Incubate 35°C ± 0.5°C for 2 ± 0.5 hours. Transfer the plates to a Whirl-Pak® bag, seal the bag, and submerge in a 44.5°C ± 0.2°C water-bath for 22 ± 2 hours.
- Data analysis: Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL
- Report results as *E. coli* CFU per 100 mL of sample.

5) *Enterococcus faecalis*

- Standard: EPA 1600 [*Enterococci* in Water by Membrane Filtration Using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI): 2006]

- SOP : SOP-BWTS-011
- Medium: mEI agar (DIFCO, Cat No 214881, Lot No 8253196)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Enterococcus faecalis* (ATCC No #19433)
- Negative control: *Escherichia coli* (ATCC No #11775)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 41°C ± 0.5°C, 24 ± 2 hours
- Data analysis: Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL
- Report results as enterococci per 100 mL of sample.

6) *Vibrio cholerae* O1, O139

- SOP: SOP-BWTS-012
- Medium: TCBS agar (DIFCO, Cat No 265020, Lot No 8021353)
- Method
 - (1) Filter sample using 0.45 µm membrane filter and then place filters on the top of plate. Incubate the plates, protected from light at 35°C ± 1°C for 24 ± 2 hours.
 - (2) Each yellow sucrose fermenting colonies are placed on Non-salt Alkaline Pepton water. Incubate the plates at 36°C ± 1 °C for 6-18 hours.
 - (3) Presumptive positive colonies are performed using slide agglutination assays by O1 and O139 antiserum for serological identification.
- Sample volume: 100 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Data analysis: Select the colonies with agglutination O1 and O139 antiserum. Calculate the number of *Vibrio cholerae* per 100 mL
- Report results as *Vibrio cholerae* O1, O139 per 100 mL of sample.

6. Validity

6.1 Water parameters

6.1.1 Measurement of water parameters must be performed at least three times.

6.1.2 Sample should be analyzed as soon as possible after arrival at the DURBAN lab.

6.2 Biological efficacy test

6.2.1 Influent condition must be appropriate for the following IMO standards;

- The organism larger than 50 µm: $\geq 10^2$ individuals/m³
- The organism between 10 to 50 µm: $\geq 10^2$ individuals / mL,

6.2.2 Average discharge results from the control water is a concentration must be more than the values in regulation D2.1;

- The organism larger than 50 µm: ≥ 10 individuals/m³
- The organism between 10 and 50 µm: ≥ 10 individuals / mL

7. Data and report

7.1 Data

7.1.1 Water parameters measurement

- 1) Measurement of water parameters (temperature, pH, DO, salinity, Turbidity) are performed at least three times using measurement equipment (Model: MS5)
- 2) Measurement of water parameters (DOC, POC) are performed at least three times using measurement equipment (Model: vario TOC cube)

$$\text{POC} = \text{TOC}-\text{DOC}$$

- 3) Measurement of water parameter(TSS) is performed at least three times.

Calculate non-filterable residue as follows:

$$\text{Non-filterable residue (mg/L)} = \frac{(A - B) \times 1000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = mL of sample filtered

7.1.2 Biological efficacy test

Result data are presented mean value using calculation method as follows;

- 1) Survival rate

$$\text{Survival rate (\%)} = \frac{N_2}{N_1} \times 100$$

where:

N_1 = number of survival organism at the beginning

N_2 = number of survival organism at the end of the selected time interval

2) Heterotrophic bacteria

Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates. And calculate count per 1 mL.

$$\text{CFU/ mL} = \frac{\text{Number of colonies}}{\text{Volume of sample (mL)}}$$

3) *Escherichia coli*

Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$E \text{ coli / 100 mL} = \frac{\text{Number of } E. \text{ coli colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

4) *Enterococcus faecalis*

Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL according to the following general formula:

$$\text{Enterococci / 100 mL} = \frac{\text{Number of enterococci colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

5) *Vibrio cholera* O1, O139

Count positive results with slide agglutination assays by O1 and O139 antiserum for serological identification. Calculate the number of *Vibrio cholera* O1, O139 per 100 mL according to the following general formula:

$$Vibrio \text{ cholera O1, O139 / 100 mL} = \frac{\text{Number of } Vibrio \text{ cholera O1, O139}}{\text{Volume of filtered sample (mL)}} \times 100$$

7.1.3 Coefficient of variation (CV)

Coefficient of variation for each replicate should be calculated as follows.

$$CV (\%) = \frac{Y}{X} \times 100$$

where:

X: The mean value for respective replicate

Y: Standard deviation for respective replicate

7.2 Report

- QA statement
- Result
- Conclusion
- Appendix

Test Report

| | |
|---------------------|---|
| Project name | Purimar™ |
| Study number | TW-Ship(5) |
| Test number | SET-10-005 |
| Port | Kaoshing (Taiwan) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | Jae Young, Baek  |

Statement of Quality Assurance

Data were reviewed by Quality Assurance Unit of DAU to assure that the study was performed in accordance with protocol and standard operating procedures (SOP) of Marine Bio-industry Development Center (MBDC). The report was an accurate reflection of the raw data generated at the MBDC. Inspection of the routine and repeated procedures that constitute the study was carried out as a continuous major phase at or about the time this study was in progress.

| Inspection Phase | Date | | |
|--|------------------|--------------------------|---------------------------|
| | Inspection | Report to Study Director | Report to Project manager |
| Test plan | Dec, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| Sampling | 1 st | Dec, 18, 2010 | Mar, 28, 2011 |
| | 2 nd | Dec, 25, 2010 | |
| Test substance | 1 st | Dec, 18, 2010 | Mar, 28, 2011 |
| | 2 nd | Dec, 25, 2010 | |
| Test procedures (Bacteria test) | 1 st | Dec, 18, 2010 | Mar, 28, 2011 |
| | 2 nd | Dec, 25, 2010 | |
| Observation and counting ($\geq 50\mu\text{m}$, $10\mu\text{m}-50\mu\text{m}$) | 1 st | Dec, 18, 2010 | Mar, 28, 2011 |
| | 2 nd | Dec, 25, 2010 | |
| Observation and counting (Bacteria test) | 1 st | Dec, 19, 2010 | Mar, 28, 2011 |
| | 2 nd | Dec, 20, 2010 | |
| | 3 rd | Dec, 26, 2010 | |
| | 4 th | Dec, 27, 2010 | |
| Raw data | Dec, 27, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| Test report | Mar, 23-25, 2011 | Mar, 28, 2011 | Mar, 28, 2011 |

Sang-Hee, Won Seungwon

Mar. 25. 2011

Sang-Hee, Won / Quality Assurance

Date

Study Personnel and Participants

The test participants recognized the study plan, manual, procedure, guide of Marine Bio-industry Development Center in performing the test.

| | Name | Date |
|---------------------------------|---|---------------|
| Study Personnel | | |
| | Jae Young, Baek (sign)  | Mar. 25. 2011 |
| | Jae Woo, Lee (sign)  | Mar. 25. 2011 |
| | Hana, Song (sign)  | Mar. 25. 2011 |
| | Eun-Jung, Jung (sign)  | Mar. 25. 2011 |
| QA/QC | | |
| | Yeon Su, Park (sign)  | Mar. 25. 2011 |
| Efficacy Testing Manager | | |
| | Byoung-Jin K. (sign)  | Mar. 25. 2011 |

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1. Results

1.1 Loading record

| | |
|-------------|---------------|
| Vessel name | HANJIN DURBAN |
| IMO number | 9375513 |

| | Water ballast tanks | | Sample WGT (Mt) | Loading (Date/Location) | Discharge (Date/Location) |
|---------|---------------------|---|-----------------|-------------------------|--------------------------------|
| Control | No.3 D/B W.B.T | S | 500 | Dec, 18 Kaoshiung | Dec, 25 21° -29.7N, 123-15E |
| Treated | No.3 D/B W.B.T | P | 500 | Dec, 18 Kaoshiung | Dec, 25 31° -51.5N, 123-15E |

1.2 Water parameters

Table 1. Water parameters at field

| Date | Sample ID | pH | Temp (°C) | Salinity (‰) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------|------------|--------------|-----------|-----------------|--------------|
| Day 0 | OwS | 7.65±0.01 | 24.75±0.00 | 35.87±0.01 | 6.30±0.17 | 4.6±0.0 | 0.904±0.000 |
| | OwM | 7.69±0.00 | 24.28±0.00 | 35.86±0.01 | 5.97±0.00 | 3.2±0.1 | 0.921±0.000 |
| | OwE | 7.65±0.01 | 24.20±0.01 | 35.88±0.01 | 5.95±0.01 | 3.4±0.1 | 0.940±0.000 |
| | T0S | 7.72±0.00 | 24.75±0.00 | 35.89±0.00 | 6.23±0.00 | 1.5±0.0 | 0.000±0.000 |
| | T0M | 7.73±0.00 | 24.56±0.01 | 35.88±0.00 | 6.09±0.01 | 3.5±0.0 | 0.000±0.000 |
| | T0E | 7.65±0.01 | 24.51±0.01 | 35.85±0.01 | 6.09±0.00 | 2.4±0.2 | 0.000±0.000 |
| Day 7 | C7S | 7.83±0.01 | 13.35±0.00 | 34.96±0.01 | 5.97±0.01 | 8.4±0.1 | 0.103±0.000 |
| | C7M | 7.73±0.01 | 13.49±0.00 | 35.52±0.00 | 5.92±0.00 | 8.2±0.0 | 0.110±0.000 |
| | C7E | 7.78±0.01 | 13.59±0.00 | 35.53±0.00 | 5.88±0.01 | 7.5±0.0 | 0.106±0.000 |
| | T7/S1 | 8.25±0.00 | 14.34±0.00 | 35.40±0.00 | 5.77±0.01 | 7.7±0.0 | 0.000±0.000 |
| | T7/S2 | 8.28±0.01 | 14.20±0.00 | 35.34±0.01 | 5.68±0.01 | 7.3±0.1 | 0.000±0.000 |
| | T7/S3 | 8.33±0.00 | 13.97±0.00 | 35.48±0.01 | 5.72±0.01 | 8.2±0.1 | 0.000±0.000 |
| | T7/M1 | 8.38±0.00 | 13.94±0.00 | 35.47±0.00 | 5.82±0.01 | 7.3±0.2 | 0.000±0.000 |
| | T7/M2 | 8.30±0.01 | 13.60±0.00 | 35.45±0.00 | 5.82±0.00 | 8.1±0.1 | 0.000±0.000 |
| | T7/M3 | 8.31±0.00 | 13.35±0.00 | 35.50±0.00 | 5.80±0.01 | 8.0±0.0 | 0.000±0.000 |
| | T7/E1 | 8.51±0.00 | 13.76±0.00 | 35.57±0.00 | 5.92±0.01 | 7.9±0.1 | 0.000±0.000 |
| | T7/E2 | 8.41±0.01 | 13.29±0.00 | 35.52±0.01 | 5.87±0.01 | 7.9±0.0 | 0.000±0.000 |
| | T7/E3 | 8.31±0.00 | 13.12±0.00 | 35.50±0.01 | 5.91±0.01 | 7.7±0.0 | 0.000±0.000 |

Data were presented as mean±S.D of three repeated measurement.

Table 2. Results of DOC, POC, TSS

| Date | Sample ID | TOC ^{a)} (mg/L) | DOC ^{b)} (mg/L) | POC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | 2.92±0.07 | 1.69±0.05 | 1.23 | 9.7±0.6 |
| | OwM | 2.98±0.12 | 1.71±0.07 | 1.28 | 8.0±0.4 |
| | OwE | 2.98±0.15 | 1.74±0.04 | 1.24 | 9.0±0.8 |

Data were presented as mean±S.D of three repeated measurement.

a) TOC: Total Organic Carbon

b) DOC: Dissolved Organic Carbon

c) POC: Particulate Organic Carbon (POC=TOC-DOC)

d) TSS: Total Suspended Solids

Table 3. TRC decay

| Date | Measurement time | Control | Treated |
|--------------|------------------|----------------|----------------|
| | | TRC con. (ppm) | TRC con. (ppm) |
| Day 0 | 21:10 | 0.04±0.01 | 2.85±0.03 |
| Day 1 | 10:00 | 0.03±0.02 | 1.88±0.04 |
| Day 2 | 10:00 | 0.04±0.01 | 1.16±0.05 |
| Day 3 | 10:00 | 0.04±0.01 | 0.64±0.06 |
| Day 4 | 10:00 | 0.02±0.00 | 0.22±0.01 |
| Day 5 | 10:00 | 0.03±0.02 | 0.03±0.01 |
| Day 6 | 10:00 | 0.05±0.02 | 0.02±0.01 |
| Day 7 | 14:00 | 0.02±0.01 | 0.03±0.01 |

Data were presented as mean±S.D of three repeated measurement.

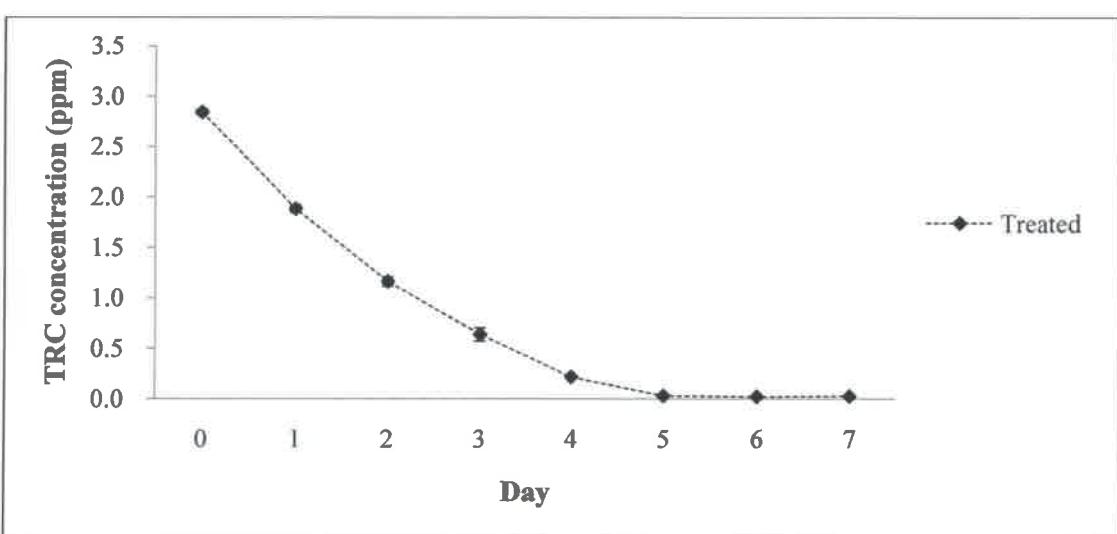


Figure 1. Change of TRC concentration of treated ballast seawater for 8 days

1.3 Influent water : Original water

1) $\geq 50 \mu\text{m}$ organismTable 4. Survival number of the organism larger than $50 \mu\text{m}$

| Sampling | Phyla/Divisions | Species | Survival number/ m^3 | Minimum Dimension (μm) |
|----------|-----------------------------|---------------------------------|---|-------------------------------------|
| Start | Podonidae | <i>Podon</i> sp. | 7280 ± 1220 | 60 ~ 120 |
| | Arthropoda | <i>Oithona</i> sp. | 3640 ± 741 | 140 ~ 200 |
| | | <i>Crustacean nauplii</i> | 93 ± 162 | 170 ~ 210 |
| | Arietellidae | <i>Paramisophria</i> sp. | 373 ± 428 | 160 ~ 200 |
| | Heterokontophyta | <i>Coscinodiscus</i> sp. | 1773 ± 428 | 200 ~ 300 |
| Middle | Calanidae | <i>Mesocalanus tenuirostris</i> | 747 ± 162 | 180 ~ 220 |
| | Podonidae | <i>Podon</i> sp. | 5693 ± 1711 | 60 ~ 120 |
| | Arthropoda | <i>Oithona</i> sp. | 5413 ± 428 | 140 ~ 200 |
| | | <i>Crustacean nauplii</i> | 93 ± 162 | 170 ~ 210 |
| | Arietellidae | <i>Paramisophria</i> sp. | 187 ± 162 | 160 ~ 200 |
| End | Heterokontophyta | <i>Coscinodiscus</i> sp. | 1400 ± 1220 | 200 ~ 300 |
| | Calanidae | <i>Mesocalanus tenuirostris</i> | 933 ± 323 | 180 ~ 220 |
| | Podonidae | <i>Podon</i> sp. | 6907 ± 2246 | 60 ~ 120 |
| | Arthropoda | <i>Oithona</i> sp. | 5040 ± 1960 | 140 ~ 200 |
| | | <i>Crustacean nauplii</i> | 373 ± 162 | 170 ~ 210 |
| Total | Heterokontophyta | <i>Coscinodiscus</i> sp. | 840 ± 485 | 200 ~ 300 |
| | Calanidae | <i>Mesocalanus tenuirostris</i> | 560 ± 0 | 180 ~ 220 |
| Total | 6 species 5 phyla/divisions | | 13782 ± 108 | - |
| | Acceptability | | Influent condition: Acceptable ($\geq 10^2$ ind./ m^3) | - |

Data were presented as mean \pm S.D of three repeated measurement.
Some species were rare, the standard deviation was high.

2) 10 µm - 50 µm organism

Table 5. Survival number of the organism between 10 to 50 µm

| Sampling | Phyla/Divisions | Species | Survival number/m ³ | Minimum Dimension (µm) |
|---------------|-----------------------------|----------------------------|---|------------------------|
| Start | Heterokontophyta | <i>Thalssiosira</i> sp. | 729 ± 59 | 15 ~ 30 |
| | | <i>Skeletonema</i> sp. | 101 ± 24 | 12 ~ 20 |
| | | <i>Chaetoceros</i> sp. | 42 ± 5 | 20 ~ 40 |
| | | <i>Melosira</i> sp. | 16 ± 3 | 15 ~ 30 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 444 ± 42 | 12 ~ 15 |
| Middle | Heterokontophyta | <i>Thalssiosira</i> sp. | 790 ± 122 | 15 ~ 30 |
| | | <i>Skeletonema</i> sp. | 84 ± 9 | 12 ~ 20 |
| | | <i>Chaetoceros</i> sp. | 37 ± 12 | 20 ~ 40 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 370 ± 46 | 12 ~ 15 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 22 ± 3 | 15 ~ 25 |
| End | Heterokontophyta | <i>Thalssiosira</i> sp. | 801 ± 18 | 15 ~ 30 |
| | | <i>Skeletonema</i> sp. | 56 ± 8 | 12 ~ 20 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 496 ± 28 | 12 ~ 15 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 11 ± 2 | 15 ~ 25 |
| | | <i>Ceratium</i> sp. | 5 ± 1 | 30 ~ 45 |
| Total | 7 species 3 phyla/divisions | | 1335 ± 66 | - |
| Acceptability | | | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |

Data were presented as mean±S.D of three repeated measurement.

Some species were rare, the standard deviation was high.

1.4 Elimination efficacy

1) $\geq 50 \mu\text{m}$ organism

Table 6. Survival number of the organism larger than $50 \mu\text{m}$

| Test substance | Sampling | Survival number/ m^3 | Acceptability | Elimination efficacy (%) |
|---|---------------|---|---|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 13907 \pm 1688 | Influent condition: Acceptable ($\geq 10^2 \text{ ind./m}^3$) | - |
| | | M ^{b)} 13720 \pm 1283 | | |
| | | E ^{c)} 13720 \pm 4311 | | |
| Control (untreated) seawater | Day 7 (C7) | S1 3553 \pm 397 | Effluent condition: Acceptable ($\geq 10 \text{ ind./m}^3$) | - |
| | | S2 4060 \pm 767 | | |
| | | E3 4510 \pm 1334 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 \pm 0 | - | 100.0 ^{d)} |
| | | M 0 \pm 0 | | |
| | | E 0 \pm 0 | | |
| | Day 7 (T7) | S1 0 \pm 0 | Effluent condition: Acceptable (<10 ind./ m^3) | 100.0 ^{e)} |
| | | S2 0 \pm 0 | | |
| | | S3 0 \pm 0 | | |
| | | M1 0 \pm 0 | | |
| | | M2 0 \pm 0 | | |
| | | M3 0 \pm 0 | | |
| | | E1 0 \pm 0 | | |
| | | E2 0 \pm 0 | | |
| | | E3 0 \pm 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean \pm S.D of three repeated measurement.

2) 10 µm - 50 µm organism

Table 7. Survival number of the organism between 10 and 50 µm

| Test substance | Sampling | Survival number/mL | | Acceptability | Elimination efficacy (%) |
|------------------------------------|---------------|--------------------|-----------|---|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 1331 ± 74 | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |
| | | M ^{b)} | 1304 ± 80 | | |
| | | E ^{c)} | 1369 ± 47 | | |
| Control (untreated) seawater | Day 7 (C7) | S | 135 ± 9 | Effluent condition: Acceptable (≥ 10 ind./mL) | - |
| | | M | 149 ± 9 | | |
| | | E | 140 ± 4 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 7 (T7) | S1 | 0 ± 0 | Effluent condition: Acceptable (<10 ind./mL) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

3) Heterotrophic bacteria

Table 8. Survival number of heterotrophic bacteria

| Test substance | Sampling | | CFU / mL | Acceptability | Elimination efficacy (%) |
|---|---------------|-----------------|------------|---------------|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 590 ± 61 | - | - |
| | | M ^{b)} | 1383 ± 156 | | |
| | | E ^{c)} | 433 ± 93 | | |
| Control (untreated) seawater | Day 7 (C7) | S | 2197 ± 224 | - | - |
| | | M | 3703 ± 225 | | |
| | | E | 2560 ± 177 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 7 (T7) | S1 | 0 ± 0 | - | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

4) *Escherichia coli***Table 9. Survival number of *Escherichia coli***

| Test substance | Sampling | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|---------------------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 46 ± 3 | - | - |
| | | M ^{b)} 54 ± 8 | | |
| | | E ^{c)} 55 ± 7 | | |
| Control (untreated) seawater | Day 7 (C7) | S 46 ± 9 | - | - |
| | | M 52 ± 10 | | |
| | | E 54 ± 7 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 ± 0 | - | 100.0 ^{d)} |
| | | M 0 ± 0 | | |
| | | E 0 ± 0 | | |
| | Day 7 (T7) | S1 0 ± 0 | Effluent condition: Acceptable (<250 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 0 ± 0 | | |
| | | S3 0 ± 0 | | |
| | | M1 0 ± 0 | | |
| | | M2 0 ± 0 | | |
| | | M3 0 ± 0 | | |
| | | E1 0 ± 0 | | |
| | | E2 0 ± 0 | | |
| | | E3 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

5) *Enterococcus faecalis***Table 10. Survival number of *Enterococcus faecalis***

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|-------------------------------------|------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 13 ± 10 | - | - |
| | | M ^{b)} | 9 ± 5 | | |
| | | E ^{c)} | 10 ± 4 | | |
| Control (untreated) seawater | Day 7 (C7) | S | 5 ± 3 | - | - |
| | | M | 6 ± 4 | | |
| | | E | 4 ± 1 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 7 (T7) | S1 | 0 ± 0 | Effluent condition: Acceptable (<100 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

6) *Vibrio cholera O1, O139***Table 11. Survival number of *Vibrio cholera O1, O139***

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 0 ± 0 | - | - |
| | | M ^{b)} | 0 ± 0 | | |
| | | E ^{c)} | 0 ± 0 | | |
| Control (untreated) seawater | Day 7 (C7) | S | 0 ± 0 | - | - |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | N.D ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 7 (T7) | S1 | 0 ± 0 | Effluent condition: Acceptable (<1 CFU/ 100ml) | N.D |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) N.D: Not detected

Data were presented as mean±S.D of three repeated measurement.

2. Validity

2.1 Water parameters measurement

- All measurements were tested more than three times.
- Water parameters were analyzed as soon as possible after sampling.

2.2 Biological efficacy test

- Influent condition in all tests was acceptable for IMO standards (MEPC58/2/4).
 - The organism larger than 50 µm: $\geq 10^2$ individuals/m³
 - The organism between 10 µm and 50 µm: $\geq 10^2$ individuals / mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| OwS | 13907 ± 1688 | 1331 ± 74 |
| OwM | 13720 ± 1283 | 1304 ± 80 |
| OwE | 13720 ± 4311 | 1369 ± 47 |

- Discharge results from the control water were a concentration more than the values in regulation D2.1.
 - The organism larger than 50 µm: ≥ 10 individuals/m³
 - The organism between 10 µm and 50 µm: ≥ 10 individuals/mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| C7S | 3553 ± 397 | 135 ± 9 |
| C7M | 4060 ± 767 | 149 ± 9 |
| C7E | 4510 ± 1334 | 140 ± 4 |

- The samples should be analysed as soon as possible after sampling.

| Date | Sample | Sampling time | Test ending time |
|-------|-------------|---------------|------------------|
| Day 0 | OwS | 19:20 | Day 1 - 01:30 |
| | OwM | 19:40 | Day 1 - 01:30 |
| | OwE | 20:00 | Day 1 - 01:30 |
| | T0S | 19:20 | Day 1 - 01:30 |
| | T0M | 19:40 | Day 1 - 01:30 |
| | T0E | 20:00 | Day 1 - 01:30 |
| Day 7 | C7S | 11:15 | 18:30 |
| | C7M | 11:53 | 18:30 |
| | C7E | 12:20 | 18:30 |
| | T7/S1, 2, 3 | 09:34 | 18:30 |
| | T7/M1, 2, 3 | 10:00 | 18:30 |
| | T7/E1, 2, 3 | 10:27 | 18:30 |

3. Conclusion

3.1 Biological efficacy in treated ballast seawater by PurimarTM BWMS

- Treated ballast seawater by PurimarTM BWMS was capable of eliminating organism larger than 50 µm with an efficiency of 100%.
- Treated ballast seawater water by PurimarTM BWMS was capable of eliminating organism between 10 and 50 µm with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating *Escherichia coli* with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating *Enterococcus faecalis* with an efficiency of 100 %.
- *Vibrio cholera* O1, O139 were not detected.
- **Therefore, treated ballast seawater by PurimarTM BWMS was capable of removing zooplankton, phytoplankton, bacteria. And PurimarTM BWMS showed discharge of treated ballast water in compliance with regulation D-2.**

4. Appendix

Appendix 1. Water parameters at field

| Date | Sample ID | R ^{a)} | pH | Temp (°C) | Salinity (‰) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------------|------|-----------|-----------------|--------------|--------------------|--------------|
| Day 0 | OwS | R1 | 7.65 | 24.75 | 35.88 | 6.29 | 4.6 | 0.904 |
| | | R2 | 7.65 | 24.75 | 35.87 | 6.00 | 4.6 | 0.904 |
| | | R3 | 7.66 | 24.75 | 35.87 | 6.29 | 4.6 | 0.904 |
| | OwM | R1 | 7.69 | 24.28 | 35.87 | 5.97 | 3.3 | 0.921 |
| | | R2 | 7.69 | 24.28 | 35.86 | 5.97 | 3.1 | 0.921 |
| | | R3 | 7.69 | 24.28 | 35.86 | 5.97 | 3.1 | 0.921 |
| | OwE | R1 | 7.65 | 24.19 | 35.87 | 5.96 | 3.3 | 0.940 |
| | | R2 | 7.65 | 24.20 | 35.88 | 5.95 | 3.4 | 0.940 |
| | | R3 | 7.66 | 24.20 | 35.88 | 5.95 | 3.4 | 0.940 |
| | T0S | R1 | 7.72 | 24.75 | 35.89 | 6.23 | 1.5 | 0.000 |
| | | R2 | 7.72 | 24.75 | 35.89 | 6.23 | 1.5 | 0.000 |
| | | R3 | 7.72 | 24.75 | 35.89 | 6.23 | 1.5 | 0.000 |
| | T0M | R1 | 7.73 | 24.56 | 35.88 | 6.09 | 3.5 | 0.000 |
| | | R2 | 7.73 | 24.56 | 35.88 | 6.09 | 3.5 | 0.000 |
| | | R3 | 7.73 | 24.55 | 35.88 | 6.10 | 3.5 | 0.000 |
| | T0E | R1 | 7.64 | 24.52 | 35.86 | 6.09 | 2.6 | 0.000 |
| | | R2 | 7.65 | 24.51 | 35.85 | 6.09 | 2.3 | 0.000 |
| | | R3 | 7.65 | 24.51 | 35.85 | 6.09 | 2.3 | 0.000 |

a) R: Replicate

Test Report

SET-10-005

| Date | Sample ID & lot No. | R ^{a)} | pH | Temp (°C) | Salinity (%) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|---------------------|-----------------|------|-----------|--------------|-----------|-----------------|--------------|
| Day 7 | C7S | R1 | 7.82 | 13.35 | 34.95 | 5.98 | 8.3 | 0.103 |
| | | R2 | 7.84 | 13.35 | 34.97 | 5.98 | 8.5 | 0.103 |
| | | R3 | 7.84 | 13.35 | 34.96 | 5.96 | 8.5 | 0.103 |
| | C7M | R1 | 7.73 | 13.49 | 35.52 | 5.92 | 8.2 | 0.110 |
| | | R2 | 7.73 | 13.49 | 35.52 | 5.92 | 8.2 | 0.110 |
| | | R3 | 7.74 | 13.49 | 35.52 | 5.92 | 8.2 | 0.110 |
| | C7E | R1 | 7.78 | 13.59 | 35.53 | 5.87 | 7.5 | 0.106 |
| | | R2 | 7.78 | 13.59 | 35.53 | 5.88 | 7.5 | 0.106 |
| | | R3 | 7.79 | 13.59 | 35.53 | 5.88 | 7.5 | 0.106 |
| | T7/S1 | R1 | 8.26 | 14.34 | 35.40 | 5.78 | 7.7 | 0.000 |
| | | R2 | 8.25 | 14.34 | 35.40 | 5.77 | 7.7 | 0.000 |
| | | R3 | 8.25 | 14.34 | 35.40 | 5.76 | 7.7 | 0.000 |
| | T7/S2 | R1 | 8.28 | 14.20 | 35.34 | 5.69 | 7.3 | 0.000 |
| | | R2 | 8.28 | 14.20 | 35.35 | 5.68 | 7.3 | 0.000 |
| | | R3 | 8.28 | 14.20 | 35.34 | 5.68 | 7.2 | 0.000 |
| | T7/S3 | R1 | 8.34 | 13.97 | 35.47 | 5.72 | 8.1 | 0.000 |
| | | R2 | 8.33 | 13.97 | 35.49 | 5.73 | 8.2 | 0.000 |
| | | R3 | 8.33 | 13.97 | 35.49 | 5.72 | 8.2 | 0.000 |
| | T7/M1 | R1 | 8.38 | 13.94 | 35.47 | 5.81 | 7.5 | 0.000 |
| | | R2 | 8.38 | 13.94 | 35.47 | 5.82 | 7.3 | 0.000 |
| | | R3 | 8.38 | 13.94 | 35.47 | 5.82 | 7.2 | 0.000 |
| | T7/M2 | R1 | 8.29 | 13.60 | 35.45 | 5.82 | 8.2 | 0.000 |
| | | R2 | 8.31 | 13.60 | 35.45 | 5.82 | 8.1 | 0.000 |
| | | R3 | 8.31 | 13.60 | 35.45 | 5.82 | 8.1 | 0.000 |
| | T7/M3 | R1 | 8.31 | 13.35 | 35.50 | 5.79 | 8.0 | 0.000 |
| | | R2 | 8.31 | 13.35 | 35.50 | 5.80 | 8.0 | 0.000 |
| | | R3 | 8.31 | 13.35 | 35.50 | 5.80 | 8.0 | 0.000 |
| | T7/E1 | R1 | 8.51 | 13.76 | 35.57 | 5.93 | 7.8 | 0.000 |
| | | R2 | 8.51 | 13.76 | 35.57 | 5.91 | 7.8 | 0.000 |
| | | R3 | 8.51 | 13.76 | 35.57 | 5.91 | 8.0 | 0.000 |
| | T7/E2 | R1 | 8.42 | 13.29 | 35.52 | 5.86 | 7.9 | 0.000 |
| | | R2 | 8.41 | 13.29 | 35.52 | 5.87 | 7.9 | 0.000 |
| | | R3 | 8.41 | 13.29 | 35.53 | 5.87 | 7.9 | 0.000 |
| | T7/E3 | R1 | 8.31 | 13.12 | 35.49 | 5.90 | 7.7 | 0.000 |
| | | R2 | 8.31 | 13.12 | 35.50 | 5.91 | 7.7 | 0.000 |
| | | R3 | 8.31 | 13.12 | 35.50 | 5.91 | 7.7 | 0.000 |

Appendix 2. Water parameters (TOC, DOC, TSS) of original water (Ow)

| Date | Sample ID | R ^{a)} | TOC ^{b)} (mg/L) | DOC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | R1 | 2.96 | 1.73 | 9.0 |
| | | R2 | 2.96 | 1.71 | 9.8 |
| | | R3 | 2.84 | 1.63 | 10.2 |
| | OwM | R1 | 3.09 | 1.78 | 8.0 |
| | | R2 | 3.00 | 1.70 | 8.4 |
| | | R3 | 2.86 | 1.64 | 7.6 |
| | OwE | R1 | 3.08 | 1.77 | 9.0 |
| | | R2 | 3.06 | 1.74 | 8.2 |
| | | R3 | 2.80 | 1.70 | 9.8 |

a) R: Replicate

b) TOC: Total Organic Carbon (TOC=DOC+POC)

c) DOC: Dissolved Organic Carbon

d) TSS: Total Suspended Solids

Appendix 3. TRC data

| Date | Group | TRC concentration (ppm) | | | Mean | S.D. ^a |
|--------------|---------|-------------------------|------|------|------|-------------------|
| Day 0 | Control | 0.03 | 0.04 | 0.04 | 0.04 | 0.01 |
| | Treated | 2.87 | 2.82 | 2.85 | 2.85 | 0.03 |
| Day 1 | Control | 0.02 | 0.05 | 0.03 | 0.03 | 0.02 |
| | Treated | 1.92 | 1.85 | 1.88 | 1.88 | 0.04 |
| Day 2 | Control | 0.03 | 0.04 | 0.04 | 0.04 | 0.01 |
| | Treated | 1.21 | 1.16 | 1.12 | 1.16 | 0.05 |
| Day 3 | Control | 0.03 | 0.05 | 0.04 | 0.04 | 0.01 |
| | Treated | 0.61 | 0.59 | 0.71 | 0.64 | 0.06 |
| Day 4 | Control | 0.02 | 0.02 | 0.02 | 0.02 | 0.00 |
| | Treated | 0.22 | 0.21 | 0.22 | 0.22 | 0.01 |
| Day 5 | Control | 0.02 | 0.06 | 0.02 | 0.03 | 0.02 |
| | Treated | 0.03 | 0.04 | 0.02 | 0.03 | 0.01 |
| Day 6 | Control | 0.06 | 0.03 | 0.05 | 0.05 | 0.02 |
| | Treated | 0.03 | 0.02 | 0.02 | 0.02 | 0.01 |
| Day 7 | Control | 0.02 | 0.02 | 0.03 | 0.02 | 0.01 |
| | Treated | 0.02 | 0.03 | 0.03 | 0.03 | 0.01 |

^a S.D: standard deviation
 * N.D: Not detected

Appendix 4. Survival number of $\geq 50\mu\text{m}$ organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number / m^3 | | | Mean | SD ^{b)} |
|-------|-----------|---------------------------------|--------------------------------|------|------|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Podon</i> sp. | 6440 | 8680 | 6720 | 7280 | 1220 |
| | | <i>Oithona</i> sp. | 4480 | 3360 | 3080 | 3640 | 741 |
| | | <i>Crustacean nauplii</i> | 0 | 0 | 280 | 93 | 162 |
| | | <i>Paramisophria</i> sp. | 280 | 840 | 0 | 373 | 428 |
| | | <i>Coscinodiscus</i> sp. | 1680 | 2240 | 1400 | 1773 | 428 |
| | | <i>Mesocalanus tenuirostris</i> | 840 | 560 | 840 | 747 | 162 |
| Day 0 | OwM | <i>Podon</i> sp. | 7560 | 4200 | 5320 | 5693 | 1711 |
| | | <i>Oithona</i> sp. | 5320 | 5880 | 5040 | 5413 | 428 |
| | | <i>Crustacean nauplii</i> | 0 | 0 | 280 | 93 | 162 |
| | | <i>Paramisophria</i> sp. | 280 | 0 | 280 | 187 | 162 |
| | | <i>Coscinodiscus</i> sp. | 840 | 2800 | 560 | 1400 | 1220 |
| | | <i>Mesocalanus tenuirostris</i> | 1120 | 560 | 1120 | 933 | 323 |
| Day 1 | OwE | <i>Podon</i> sp. | 4760 | 6720 | 9240 | 6907 | 2246 |
| | | <i>Oithona</i> sp. | 4200 | 3640 | 7280 | 5040 | 1960 |
| | | <i>Crustacean nauplii</i> | 280 | 560 | 280 | 373 | 162 |
| | | <i>Coscinodiscus</i> sp. | 280 | 1120 | 1120 | 840 | 485 |
| | | <i>Mesocalanus tenuirostris</i> | 560 | 560 | 560 | 560 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 5. Survival number of $\geq 50\mu\text{m}$ organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number /m ³ | | | Mean | SD ^{b)} |
|--------------|-----------|--------------------|---------------------------------|------|------|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 7 | C7S | <i>Oithona</i> sp. | 3900 | 3640 | 3120 | 3553 | 397 |
| | C7M | <i>Oithona</i> sp. | 3480 | 3770 | 4930 | 4060 | 767 |
| | C7E | <i>Oithona</i> sp. | 2970 | 5280 | 5280 | 4510 | 1334 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 6. Survival number of $\geq 50\mu\text{m}$ organism in treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|----------------|-------------------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | TOS | - | 0 | 0 | 0 | 0 | 0 |
| | TOM | - | 0 | 0 | 0 | 0 | 0 |
| | T0E | - | 0 | 0 | 0 | 0 | 0 |
| Day 7 | T7/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 7. Survival number of 10 μm - 50 μm organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|-------|-----------|----------------------------|----------------------|-----|-----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Thalassiosira</i> sp. | 673 | 790 | 723 | 729 | 59 |
| | | <i>Skeletonema</i> sp. | 98 | 79 | 126 | 101 | 24 |
| | | <i>Chaetoceros</i> sp. | 36 | 45 | 44 | 42 | 5 |
| | | <i>Melosira</i> sp. | 13 | 19 | 16 | 16 | 3 |
| | | <i>Tetraselmis</i> sp. | 452 | 481 | 398 | 444 | 42 |
| Day 0 | OwM | <i>Thalassiosira</i> sp. | 832 | 653 | 886 | 790 | 122 |
| | | <i>Skeletonema</i> sp. | 88 | 91 | 74 | 84 | 9 |
| | | <i>Chaetoceros</i> sp. | 51 | 31 | 29 | 37 | 12 |
| | | <i>Tetraselmis</i> sp. | 327 | 418 | 365 | 370 | 46 |
| | | <i>Protoperidinium</i> sp. | 25 | 23 | 19 | 22 | 3 |
| Day 0 | OwE | <i>Thalassiosira</i> sp. | 821 | 785 | 796 | 801 | 18 |
| | | <i>Skeletonema</i> sp. | 63 | 57 | 48 | 56 | 8 |
| | | <i>Tetraselmis</i> sp. | 521 | 465 | 502 | 496 | 28 |
| | | <i>Protoperidinium</i> sp. | 10 | 13 | 11 | 11 | 2 |
| | | <i>Ceratium</i> sp. | 4 | 6 | 6 | 5 | 1 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 8. Survival number of 10µm - 50µm organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|--------------|-----------|--------------------------|----------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 7 | C7S | <i>Thalassiosira</i> sp. | 64 | 79 | 71 | 71 | 8 |
| | | <i>Chaetoceros</i> sp. | 2 | 4 | 5 | 4 | 2 |
| | | <i>Tetraselmis</i> sp. | 60 | 61 | 59 | 60 | 1 |
| | C7M | <i>Thalassiosira</i> sp. | 58 | 61 | 60 | 60 | 2 |
| | | <i>Chaetoceros</i> sp. | 6 | 1 | 4 | 4 | 3 |
| | | <i>Tetraselmis</i> sp. | 84 | 79 | 95 | 86 | 8 |
| | C7E | <i>Thalassiosira</i> sp. | 70 | 77 | 81 | 76 | 6 |
| | | <i>Tetraselmis</i> sp. | 66 | 58 | 61 | 62 | 4 |
| | | <i>Ceratium</i> sp. | 3 | 2 | 2 | 2 | 1 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

**Appendix 9. Survival number of 10 μm - 50 μm organism in treated ballast seawater
by treatment of Purimar™ BWMS**

| Date | Sample ID | Classification | Survival number/mL | | | Mean | SD ^{b)} |
|-------|-----------|----------------|--------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | T0S | - | 0 | 0 | 0 | 0 | 0 |
| | T0M | - | 0 | 0 | 0 | 0 | 0 |
| | T0E | - | 0 | 0 | 0 | 0 | 0 |
| Day 7 | T7/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T7/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 10. Number of heterotrophic bacteria in original water, control (untreated) seawater and treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | CFU/mL | | | Mean | SD ^{b)} | |
|-------|-----------|------------------|-------|-------|-------|------------------|-----|
| | | R1 ^{a)} | R2 | R3 | | | |
| Day 0 | Ow | Start | 550 | 560 | 660 | 590 | 61 |
| | | Middle | 1,240 | 1,360 | 1,550 | 1,383 | 156 |
| | | End | 330 | 460 | 510 | 433 | 93 |
| | T0 | Start | 0 | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 | 0 |
| Day 7 | C7 | S | 2,000 | 2,150 | 2,440 | 2,197 | 224 |
| | | M | 3,510 | 3,650 | 3,950 | 3,703 | 225 |
| | | E | 2,400 | 2,530 | 2,750 | 2,560 | 177 |
| | T7 | S1 | 0 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 11. Number of *Escherichia coli* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 42 | 48 | 48 | 46 |
| | | Middle | 46 | 56 | 61 | 54 |
| | | End | 49 | 53 | 63 | 55 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day 7 | C7 | S | 39 | 42 | 56 | 46 |
| | | M | 43 | 50 | 62 | 52 |
| | | E | 49 | 51 | 62 | 54 |
| | T7 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 12. Number of *Enterococcus faecalis* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} | |
|-------|-----------|------------------|----|----|------|------------------|----|
| | | R1 ^{a)} | R2 | R3 | | | |
| Day 0 | Ow | Start | 6 | 8 | 24 | 13 | 10 |
| | | Middle | 5 | 7 | 15 | 9 | 5 |
| | | End | 5 | 11 | 13 | 10 | 4 |
| | T0 | Start | 0 | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 | 0 |
| | C7 | S | 2 | 6 | 7 | 5 | 3 |
| | | M | 3 | 6 | 10 | 6 | 4 |
| | | E | 3 | 4 | 5 | 4 | 1 |
| Day 7 | T7 | S1 | 0 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 | 0 |
| | T7 | M1 | 0 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

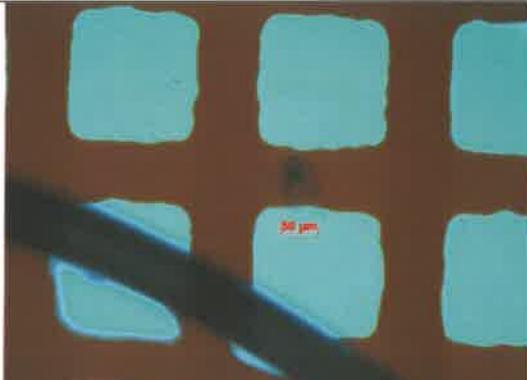
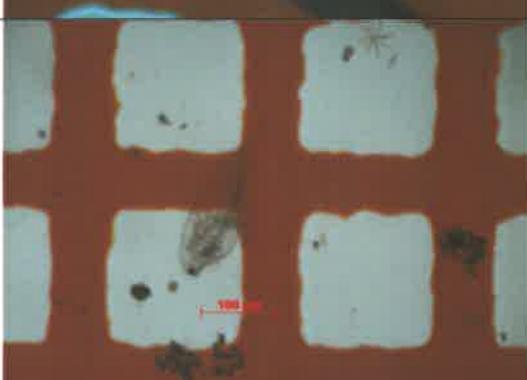
Appendix 13. Number of *Vibrio cholera* O1, O139 in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day 7 | C7 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 0 | 0 |
| | | E | 0 | 0 | 0 | 0 |
| | S1 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | T7 | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | E1 | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

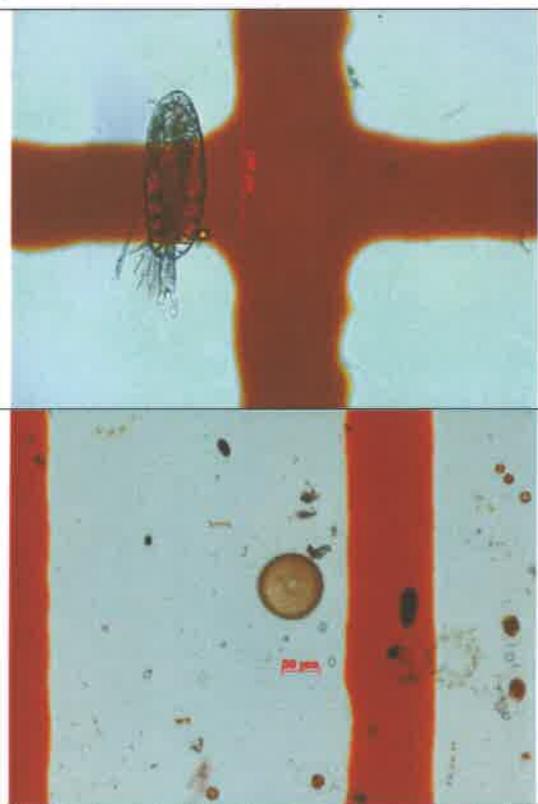
a) R: Replicate

b) S.D: Standard deviation

Appendix 14. The microscope image of $\geq 50\mu\text{m}$ in original water (Day 0)

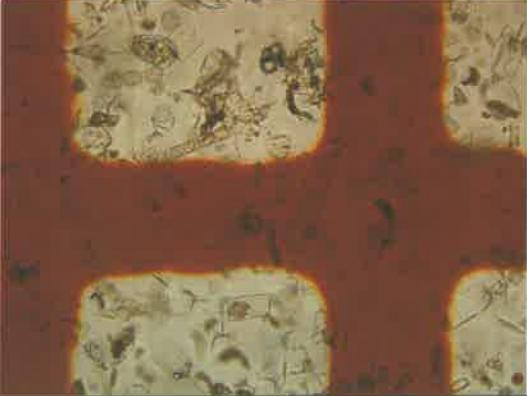
| Specific Name | Original water |
|----------------------------------|--|
| <i>Podon</i> sp. |  |
| <i>Oithona</i> sp. |  |
| <i>Crustacean nauplii</i> |  |
| <i>Mesocalanus tenuirocornis</i> |  |

Paramisophria sp.

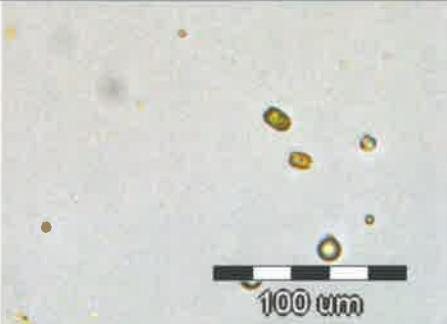
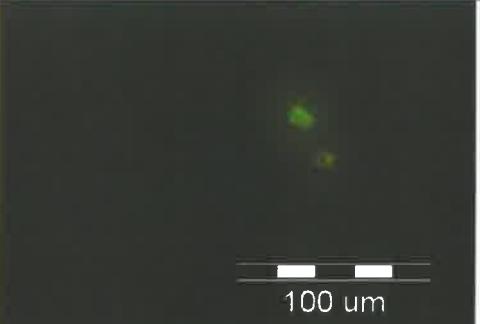
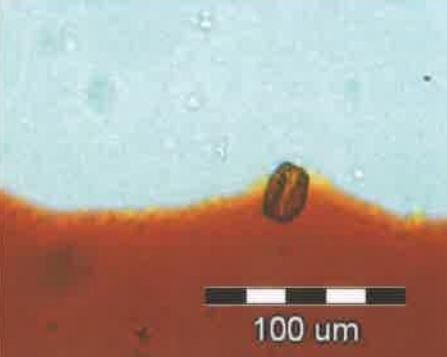
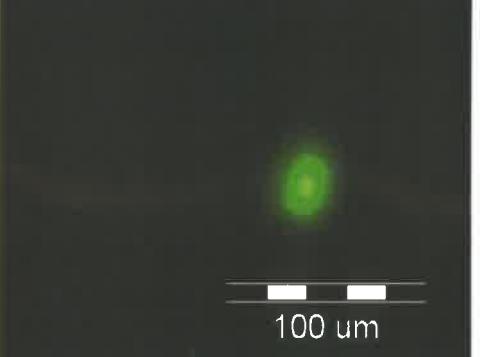
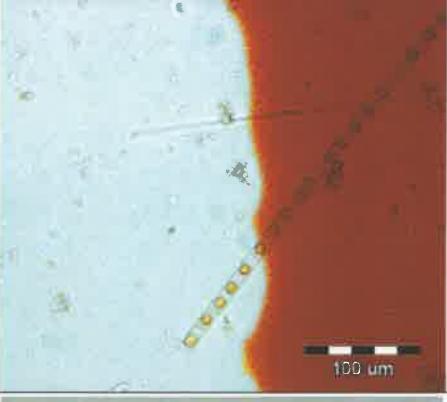
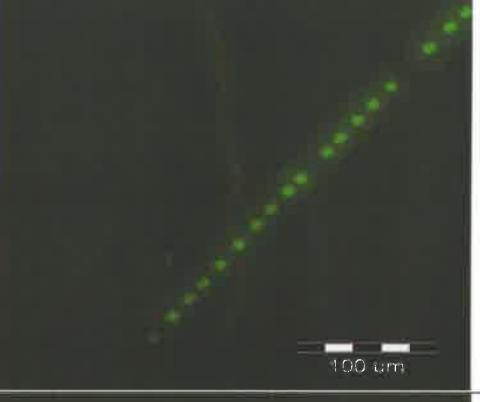
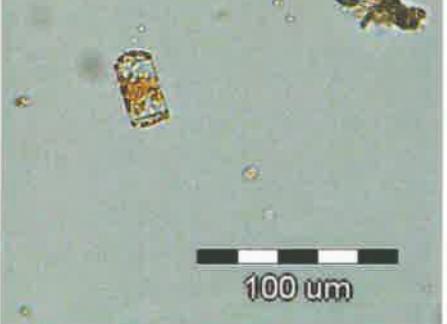
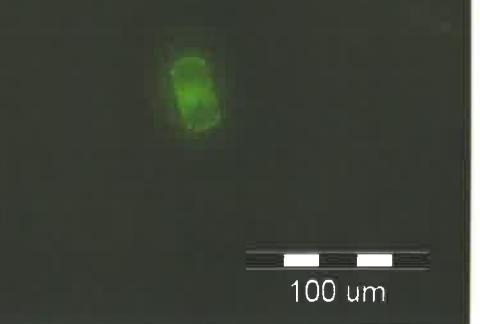


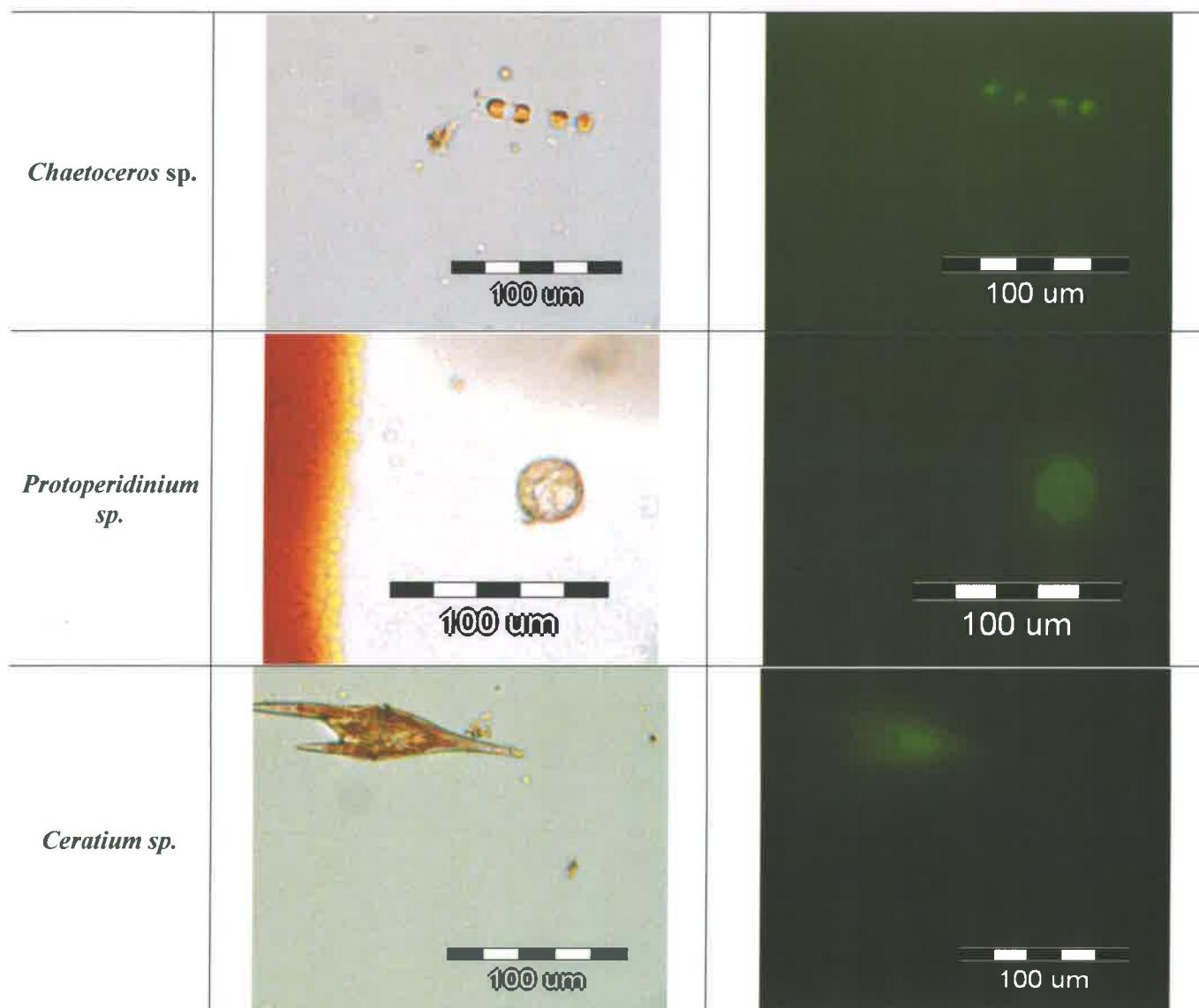
Coscinodiscus sp.

Appendix 15. The microscope image of $\geq 50\mu\text{m}$ in treated ballast seawater by treatment of PurimarTM BWMS (Day 0)

| Specific Name | Control (untreated) seawater |
|---------------|--|
| |  |

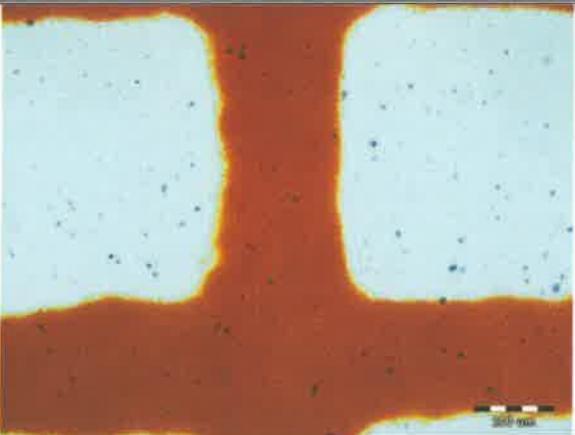
Appendix 16. The microscope image of $10\mu\text{m}$ - $50\mu\text{m}$ in original water (Day 0)

| Specific Name | Original water | |
|----------------------------|---|---|
| | Optical | Fluorescence |
| <i>Tetraselmis suecica</i> |  |  |
| <i>Thalassiosira sp.</i> |  |  |
| <i>Skeletonema sp.</i> |  |  |
| <i>Melosira sp.</i> |  |  |



Magnification : 200

Appendix 17. The microscope image of $10\mu\text{m}$ - $50\mu\text{m}$ in treated ballast seawater by treatment of PurimarTM BWMS (Day 0)

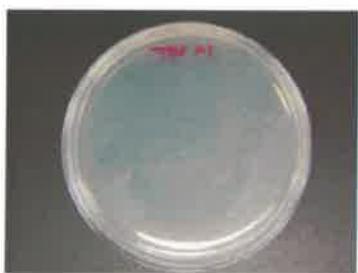
| Specific Name | Treated ballast seawater | |
|---------------|---|--|
| | Optical | Fluorescence |
| - |  |  |

Magnification : 200

Appendix 18. The image of heterotrophic bacteria: Day 0

Treated ballast seawater

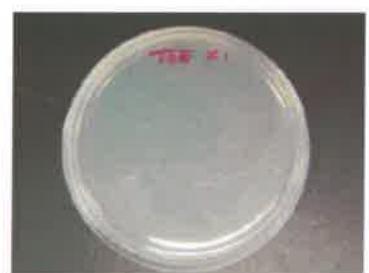
Start



Middle



End



Original water

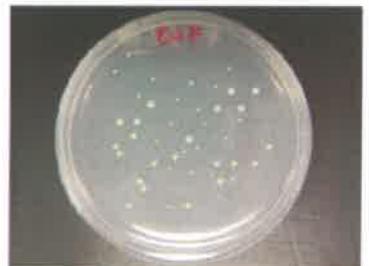
Start

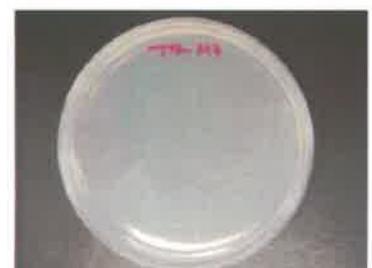
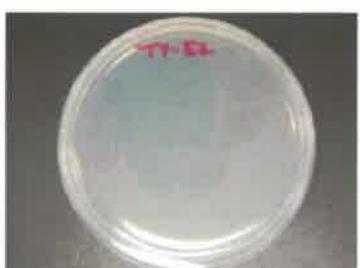


Middle



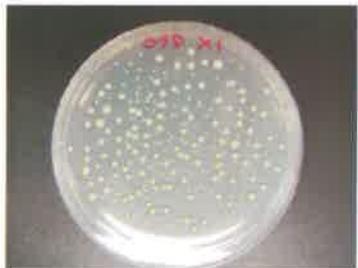
End



Appendix 19. The image of heterotrophic bacteria: Day 7**Treated ballast seawater****Strat(1)****Strat(2)****Strat(3)****Middle(1)****Middle(2)****Middle(3)****End(1)****End(2)****End(3)**

Control (untreated) seawater

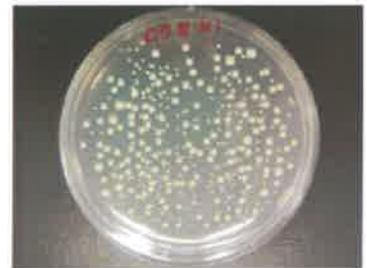
Start



Middle



End



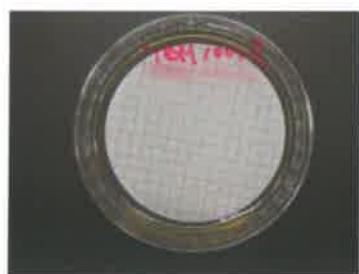
Appendix 20. The image of *Escherichia coli*: Day 0

Treated ballast seawater

Start



Middle

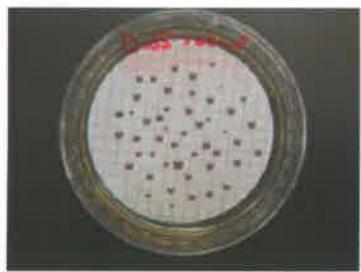


End

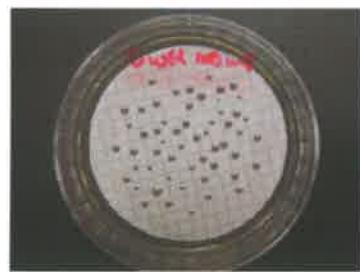


Original water

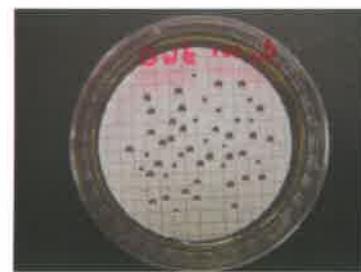
Start



Middle



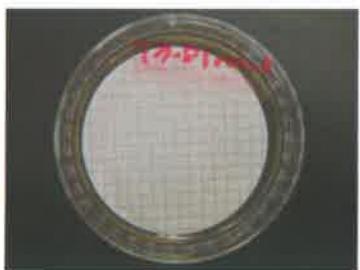
End



Appendix 21. The image of *Escherichia coli*: Day 7

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



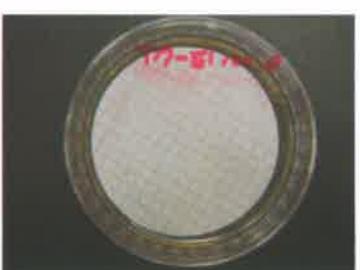
Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start

Middle

End



Appendix 22. The image of *Enterococcus faecalis*: Day 0

Treated ballast seawater

Start



Middle



End

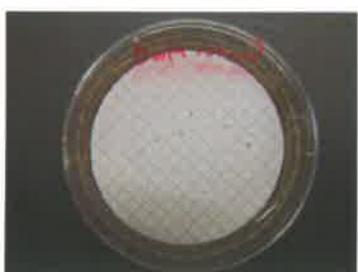


Original water

Start



Middle



End



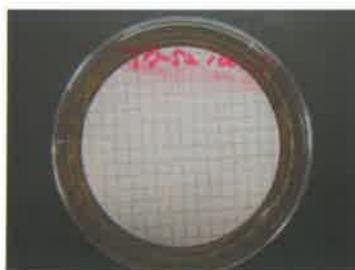
Appendix 23. The image of *Enterococcus faecalis*: Day 7

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



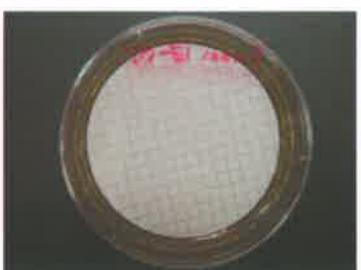
Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 24. The image of *Vibrio cholera* O1, O139: Day 0

Original water

Start

Middle

End



Appendix 25. The image of *Vibrio cholera* O1, O139: Day 7

Control (untreated) seawater

Start

Middle

End



5. Attachment

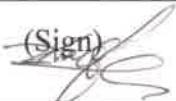
- 5.1 Loading record
- 5.2 Sampling check & custody sheet
- 5.3 Freezing keeping sheet
- 5.4 Chain of custody record
- 5.5 Sample receipt form
- 5.6 Water parameter measurement sheet (I)
- 5.7 TOC measurement sheet
- 5.8 TSS(Total suspended solid) measurement sheet
- 5.9 TRC measurement sheet(For field)
- 5.10 Microbiology test sheet
- 5.11 Test results sheet with microbiology
- 5.12 Vibrio cholera O1, O139
- 5.13 Test results sheet – Counting
- 5.14 Test results sheet – Classification(II)

첨부(3)

시험번호 : SET-10-006

시험항구 : Busan(Korea)

Test Plan

| | |
|---------------------|---|
| Project name | Purimar™ |
| Study number | TW-Ship(6) |
| Test number | SET-10-006 |
| Port | Busan (Korea) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | Jae Young, Park  (Sign) |

1. Test title

Efficacy test of Purimar™ Ballast Water Management System (shipboard scale)

2. Test purpose

- 2.1 The objective of the present test is to evaluate the efficacy of Purimar™ Ballast Water Management System(BWMS).
- 2.2 We will determine elimination efficacy of organism lager than 50 µm, organism between 10 and 50 µm and bacteria by treatment of Purimar™ BWMS.

3. Schedule

3.1 The expecting port of shipboard test : Busan(Korea)

3.2 Equipment usage(treatment) schedule

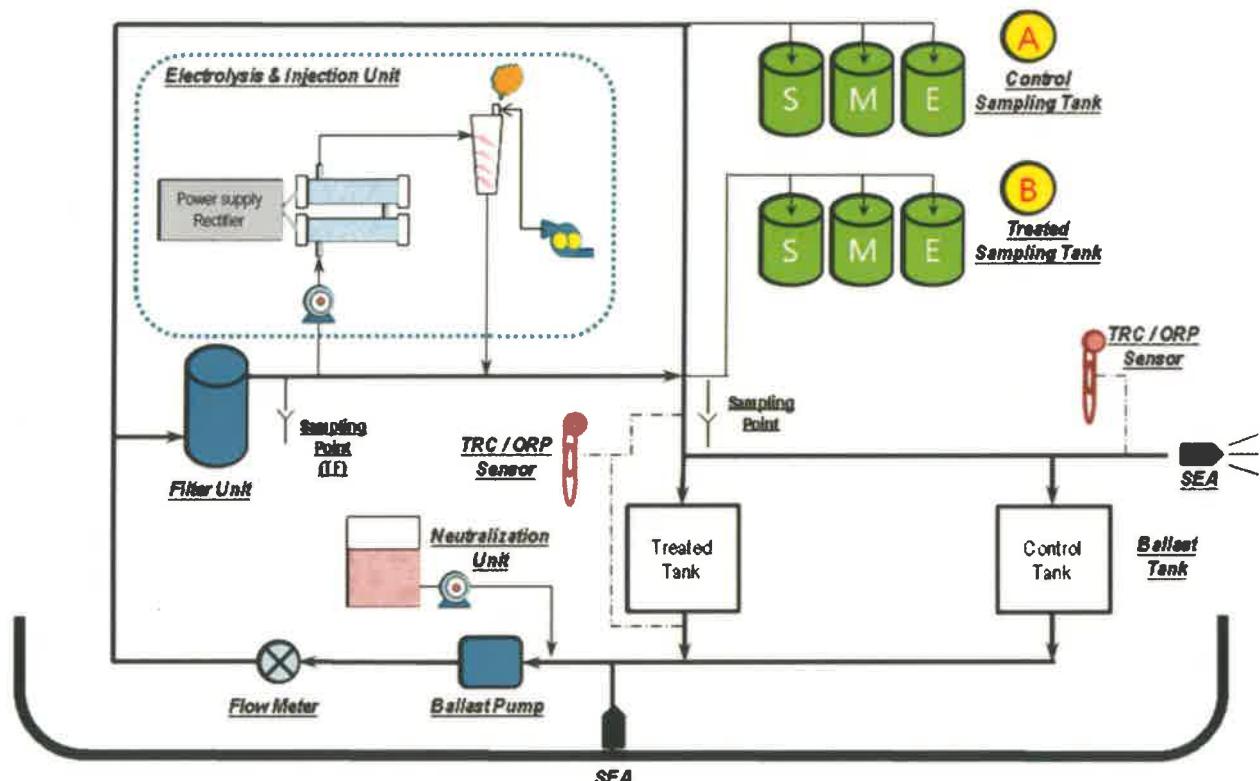
| Test time | Date | |
|-----------|--------------------|--|
| | Purimar™ Treatment | Neutralizing agent treatment or Deballasting |
| Day 0 | Dec 26, 2010 | - |
| Day 6 | - | Jan 01, 2011 |

3.3 Test schedule

| Test time | Date | | | | |
|-----------|---|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| | *Water parameters * $\geq 50 \mu\text{m}$ Organisms * 10~50 μm Organisms | *Coliform *Enterococcus group | * <i>V. cholera</i> | * Heterotrophic bacteria (Marine) | |
| Day 0 | Dec 26, 2010 | Dec 26, 2010 ~ Dec 27, 2010 | Dec 26, 2010 ~ Dec 28, 2010 | Dec 26, 2010 ~ Dec 29, 2010 | Dec 26, 2010 ~ Dec 29, 2010 |
| Day 6 | Jan 01, 2011 | Jan 01, 2011 ~ Jan 01, 2011 | Jan 01, 2011 ~ Jan 02, 2011 | Jan 01, 2011 ~ Jan 02, 2011 | Jan 01, 2011 ~ Jan 03, 2011 |

4. Sampling procedures

4.1 Sampling point



| Sampling point | Day | Sample name | Parameters |
|----------------|-------|---|--|
| A | Day 0 | Original water / Control (untreated) seawater | Water parameters ^{a)} Organism ^{b)} Bacteria ^{c)} |
| B | Day 6 | Treated ballast seawater | Water parameters ^{d)} Organism ^{b)} Bacteria ^{c)} |

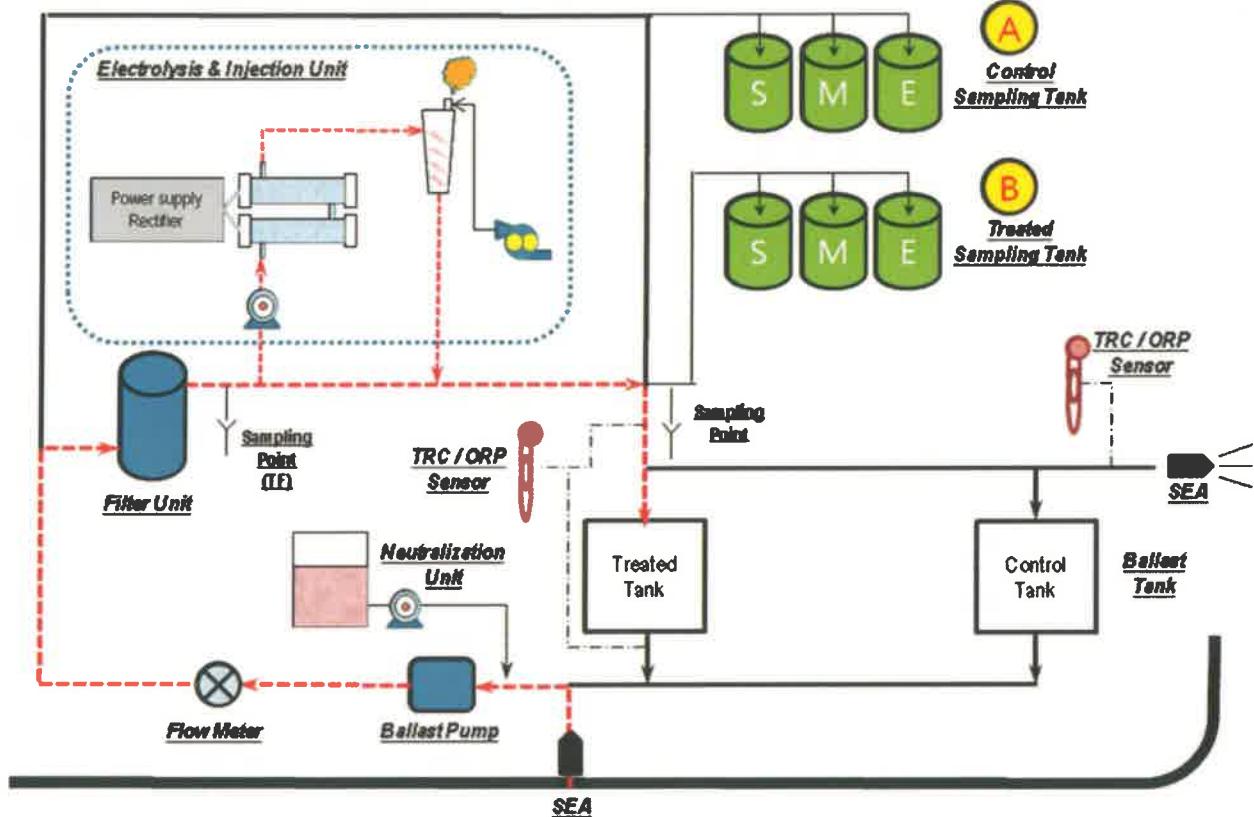
a) Water parameters: pH, Temperature, Salinity, DO, Turbidity, DOC, POC, TSS

b) Organism: $\geq 50 \mu\text{m}$ Organisms, $10\mu\text{m} - 50\mu\text{m}$ Organisms

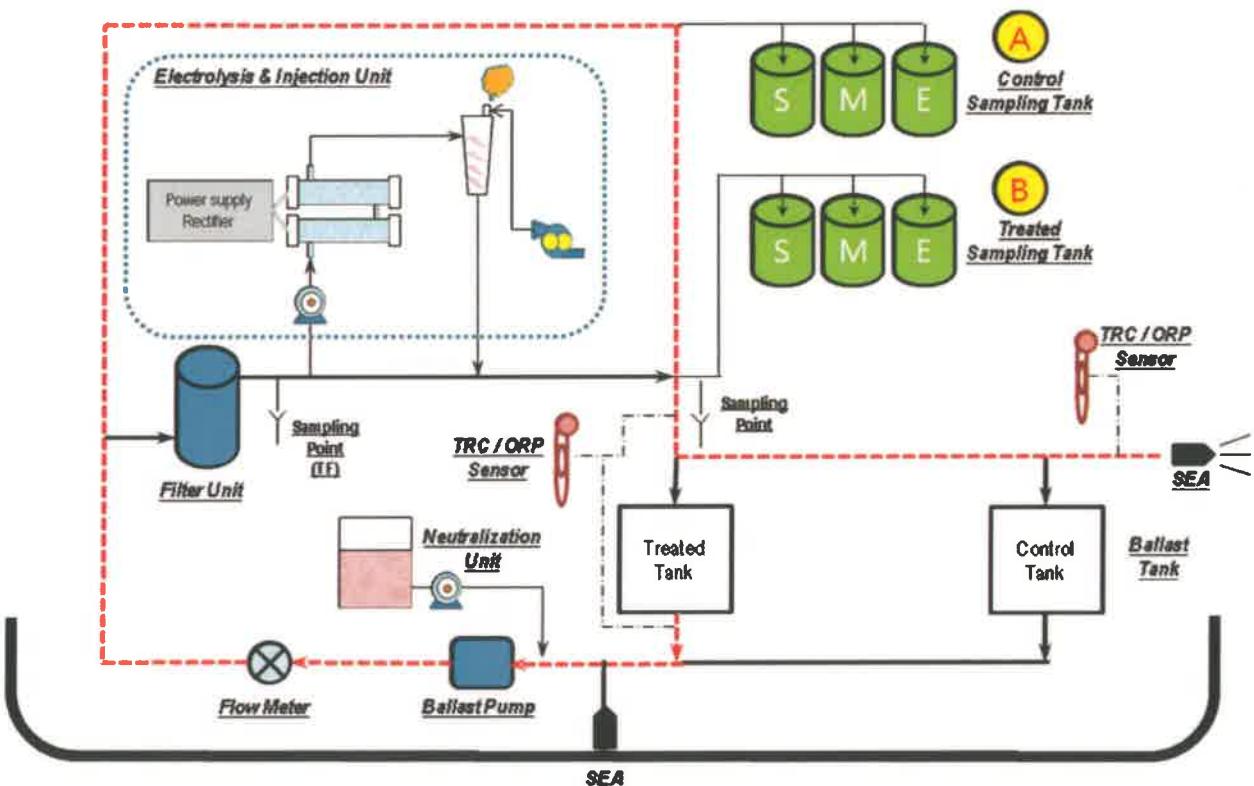
c) Bacteria: Heterotrophic bacteria, Coliform, Enterococcus group, *V. cholera*

d) Water parameters: pH, Temperature, Salinity, DO, Turbidity

4.2 Ballasting mode



4.3 Deballasting mode



4.4 Sampling procedures

- Collection and handling of field samples from the HANJIN “the DURBAN” will be undertaken by a team from MBDC and NLP Co., Ltd, using standard water sample collection methods and in accordance with the G8 Guidelines. Standard operating procedure (SOP) - SOP-BWMS-023 is employed to provide consistency and reproducibility to the sampling methods used by field personnel.
- Water samples will be taken from both the control (untreated) and treated tanks at two times intervals following treatment – at the ballasting (immediately after treatment, day 0), and at the discharge (deballasting or neutralizing agent treatment after seven days, day 6), and identified as numbered sampling points.
- All sampling equipments, apparatus and containers are prepared in accordance with EPA’s Coastal 2000 Field Operation Manual. They are packed into exclusive cases.
- Water samples should be directly taken full up in a sample bottles after washing by sample water. When sample bottles are pre-preserved, the bottles should not be rinsed but be filled once with sample.
- Water parameters of samples are analyzed as soon as possible after collection at a field. The collected samples are transported to the laboratory in the DURBAN for the analysis.
- When the samples are arrived to laboratory, laboratory personnel receive the samples and entered the samples into the laboratory. The laboratory custodian will open the sample and carefully check the contents for evidence of leakage
- Sample handling will be performed so as to collect, store, submit to the laboratory and analyze representative samples using methods as specified in the test plans.

4.5 Test substances of Day 0

- Influent water (Original water)

| Parameter | Sampling point | Sample ID & Lot No. |
|------------------|----------------|---------------------|
| Organisms | A | OwS-1226 |
| Bacteria | | OwM-1226 |
| Water parameters | | OwE-1226 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T0S-1226 |
| Heterotrophic bacteria | | T0M-1226 |
| Water parameters | | T0E-1226 |

4.6 Test substances of Day 5

- Control (untreated) seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------|---------------|---------------------|
| Organisms | A | C6S-0101 |
| Bacteria | | C6M-0101 |
| Water parameters | | C6E-0101 |

- Treated ballast seawater

| Parameter | Sampling site | Sample ID & Lot No. |
|------------------------|---------------|---------------------|
| Organisms | B | T6/S1, 2, 3-0101 |
| Heterotrophic bacteria | | T6/M1, 2, 3-0101 |
| Water parameters | | T6/E1, 2, 3-0101 |

5. Test procedures

5.1 Test design

| | |
|--------------------------|--|
| Test system | Purimar™ (shipboard scale) |
| Test substance | Original water: 100% Control (untreated) seawater: 100% Treated ballast seawater: 100% |
| Dilution water | Filtered natural seawater (FNS) |
| TRC concentration | Control: 0.0 ppm Treated: 3.0 ppm(± 0.5 ppm) |
| Sampling time | Day 0, 6 |

5.2 Test method

5.2.1 Water parameters measurement

- 1) Water parameters (temperature, pH, DO, salinity, turbidity) of samples at ship(HANJIN DURBAN) are measured using an MS5 according to SOP-BWMS-022.
- 2) Water parameters (DOC, POC) of samples at MBDC lab. are measured using vario TOC cube according to SOP-BWMS-021.
- 3) Water parameter (TSS) of samples at MBDC lab. is measured according to SOP-BWMS-005.

5.2.2 Biological efficacy test

- 1) ≥ 50µm organism
 - ① Concentration
 - Sample can be concentrated with 32 µm sieve.
 - After concentration, wash with filtered natural seawater to gather organisms.
 - Concentrated sample transfer into glass beaker and fill up to 100 mL with filtered natural seawater.
 - ② Analysis
 - General method
 - Analysis under stereo microscope with dark field (alive: movement /dead: lack of movement).
 - 1 ~ 20 mL of concentration sample place on a counting chamber (sedgewick-Rafter cell or Bogorov counting chamber).
 - The number of observations must be more than three.

- Analysis by process of dyeing
 - Making of FDA Stock solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
 - Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
 - Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
 - 1 mL of dyed sample place on a counting chamber.
 - Waiting for 10 minutes for cell staining.
 - Turn on mercury burner of microscope and apply to a fluorescent filter.
 - Viable cells represent green color.
 - Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

2) 10 μ m - 50 μ m organism

① General method

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- 0.1 mL of fixed sample place on a counting chamber (sedgewick-Rafter cell).
- Waiting for 5 minutes for cell sinking.
- The number of observations must be more than three.

② Staining

- Sample can be fixed with a formalin solution (final concentration 0.1%).
- Making of FDA Stock Solution (1 mL)
1 mL of 100 % DMSO solution inject to a 5 mg of FDA, which keep frozen until use.
- Making of Working Solution
Prepared by diluting the primary DMSO solution 100 times with stock solution.
- Each sample stained by adding 0.1 mL of the working solution to 3 mL sample.
(final concentration: 1.7 μ L/mL FDA)
- Dyed sample place on a counting chamber.
- Waiting for 10 minutes for cell staining.
- Turn on mercury burner of microscope and apply to a fluorescent filter.
- Viable cells represent green color.
- Sample could be saved for up to 90 minutes without risking significant fluorescent degradation.

3) Heterotrophic bacteria

- Standard: APHA 9215 (Heterotrophic plate count: 2005)
- SOP: SOP-BWTS-009
- Medium: Marine agar (DIFCO, Cat No 212185, Lot No 8129045)
- Method: Sample smear on the agar plate.
- Sample volume: 0.1 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 25°C, 3 days.
- Data analysis: Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates promptly after incubation. And calculate count per 1 mL.

4) *Escherichia coli*

- Standard: EPA 1603 [*Escherichia coli (E. coli)* in Water by Membrane Filtration Using Modified membrane - Thermo tolerant *Escherichia coli* Agar (Modified mTEC) : 2006]
- SOP: SOP-BWTS-010
- Medium: mTEC agar (DIFCO, Cat No 214880, Lot No 8171842)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Escherichia coli* (ATCC No #11775)
- Negative control: *Enterococcus faecalis* (ATCC No #19433)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: Incubate 35°C ± 0.5°C for 2 ± 0.5 hours. Transfer the plates to a Whirl-Pak® bag, seal the bag, and submerge in a 44.5°C ± 0.2°C water-bath for 22 ± 2 hours.
- Data analysis: Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL
- Report results as *E. coli* CFU per 100 mL of sample.

5) *Enterococcus faecalis*

- Standard: EPA 1600 [*Enterococci* in Water by Membrane Filtration Using membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI): 2006]

- SOP : SOP-BWTS-011
- Medium: mEI agar (DIFCO, Cat No 214881, Lot No 8253196)
- Method: Filter sample using 0.45 µm membrane filter and then place filters on the top of plate.
- Sample volume: 100 mL
- Positive control: *Enterococcus faecalis* (ATCC No #19433)
- Negative control: *Escherichia coli* (ATCC No #11775)
- Dilution water: Filtered natural seawater (Autoclaved)
- Incubation: 41°C ± 0.5°C, 24 ± 2 hours
- Data analysis: Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL
- Report results as enterococci per 100 mL of sample.

6) *Vibrio cholerae* O1, O139

- SOP: SOP-BWTS-012
- Medium: TCBS agar (DIFCO, Cat No 265020, Lot No 8021353)
- Method
 - (1) Filter sample using 0.45 µm membrane filter and then place filters on the top of plate. Incubate the plates, protected from light at 35°C ± 1°C for 24 ± 2 hours.
 - (2) Each yellow sucrose fermenting colonies are placed on Non-salt Alkaline Pepton water. Incubate the plates at 36°C ± 1 °C for 6-18 hours.
 - (3) Presumptive positive colonies are performed using slide agglutination assays by O1 and O139 antiserum for serological identification.
- Sample volume: 100 mL
- Positive control: None
- Negative control: Filtered natural seawater (Autoclaved)
- Dilution water: Filtered natural seawater (Autoclaved)
- Data analysis: Select the colonies with agglutination O1 and O139 antiserum. Calculate the number of *Vibrio cholerae* per 100 mL
- Report results as *Vibrio cholerae* O1, O139 per 100 mL of sample.

6. Validity

6.1 Water parameters

6.1.1 Measurement of water parameters must be performed at least three times.

6.1.2 Sample should be analyzed as soon as possible after arrival at the DURBAN lab.

6.2 Biological efficacy test

6.2.1 Influent condition must be appropriate for the following IMO standards;

- The organism larger than 50 µm: $\geq 10^2$ individuals/m³
- The organism between 10 to 50 µm: $\geq 10^2$ individuals / mL,

6.2.2 Average discharge results from the control water is a concentration must be more than the values in regulation D2.1;

- The organism larger than 50 µm: ≥ 10 individuals/m³
- The organism between 10 and 50 µm: ≥ 10 individuals / mL

7. Data and report

7.1 Data

7.1.1 Water parameters measurement

- 1) Measurement of water parameters (temperature, pH, DO, salinity, Turbidity) are performed at least three times using measurement equipment (Model: MS5)
- 2) Measurement of water parameters (DOC, POC) are performed at least three times using measurement equipment (Model: vario TOC cube)

$$\text{POC}=\text{TOC}-\text{DOC}$$

- 3) Measurement of water parameter(TSS) is performed at least three times.

Calculate non-filterable residue as follows:

$$\text{Non-filterable residue (mg/L)} = \frac{(A - B) \times 1000}{C}$$

where:

A = weight of filter (or filter and crucible) + residue in mg

B = weight of filter (or filter and crucible) in mg

C = mL of sample filtered

7.1.2 Biological efficacy test

Result data are presented mean value using calculation method as follows;

- 1) Survival rate

$$\text{Survival rate (\%)} = \frac{N_2}{N_1} \times 100$$

where:

N_1 = number of survival organism at the beginning

N_2 = number of survival organism at the end of the selected time interval

2) Heterotrophic bacteria

Select the plate with the number of colonies in the acceptable range (30-300 colonies) and count all colonies on selected plates. And calculate count per 1 mL.

$$\text{CFU/ mL} = \frac{\text{Number of colonies}}{\text{Volume of sample (mL)}}$$

3) *Escherichia coli*

Select the plate with 20-80 magenta or red colonies, and calculate the number of *E. coli* per 100 mL according to the following general formula:

$$E \text{ coli / 100 mL} = \frac{\text{Number of } E. \text{ coli colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

4) *Enterococcus faecalis*

Select the plate with 20-60 colonies (regardless of colony color) with a blue halo. Calculate the number of enterococci per 100 mL according to the following general formula:

$$\text{Enterococci / 100 mL} = \frac{\text{Number of enterococci colonies}}{\text{Volume of filtered sample (mL)}} \times 100$$

5) *Vibrio cholera* O1, O139

Count positive results with slide agglutination assays by O1 and O139 antiserum for serological identification. Calculate the number of *Vibrio cholera* O1, O139 per 100 mL according to the following general formula:

$$Vibrio \text{ cholera O1, O139 / 100 mL} = \frac{\text{Number of } Vibrio \text{ cholera O1, O139}}{\text{Volume of filtered sample (mL)}} \times 100$$

7.1.3 Coefficient of variation (CV)

Coefficient of variation for each replicate should be calculated as follows.

$$CV (\%) = \frac{Y}{X} \times 100$$

where:

X: The mean value for respective replicate

Y: Standard deviation for respective replicate

7.2 Report

- QA statement
- Result
- Conclusion
- Appendix

Test Report

| | |
|---------------------|---|
| Project name | Purimar™ |
| Study number | TW-Ship(6) |
| Test number | SET-10-006 |
| Port | Busan (Korea) |
| Title | Efficacy test of Purimar™ Ballast Water Management System (shipboard scale) |
| Prepared by | Jae Young Back  <small>(Sign)</small> |

Statement of Quality Assurance

Data were reviewed by Quality Assurance Unit of DAU to assure that the study was performed in accordance with protocol and standard operating procedures (SOP) of Marine Bio-industry Development Center (MBDC). The report was an accurate reflection of the raw data generated at the MBDC. Inspection of the routine and repeated procedures that constitute the study was carried out as a continuous major phase at or about the time this study was in progress.

| Inspection Phase | | Date | | |
|--|-----------------|------------------|--------------------------|---------------------------|
| | | Inspection | Report to Study Director | Report to Project manager |
| Test plan | | Dec, 13, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| Sampling | 1 st | Dec, 26, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Jan, 01, 2011 | | |
| Test substance | 1 st | Dec, 26, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Jan, 01, 2011 | | |
| Test procedures (Bacteria test) | 1 st | Dec, 26, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Jan, 01, 2011 | | |
| Observation and counting (≥50µm, 10µm-50µm) | 1 st | Dec, 26, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Jan, 01, 2011 | | |
| Observation and counting (Bacteria test) | 1 st | Dec, 27, 2010 | Mar, 28, 2011 | Mar, 28, 2011 |
| | 2 nd | Dec, 28, 2010 | | |
| | 3 rd | Jan, 01, 2011 | | |
| | 4 th | Jan, 02, 2011 | | |
| Raw data | | Jan, 07, 2011 | Mar, 28, 2011 | Mar, 28, 2011 |
| Test report | | Mar, 23-25, 2011 | Mar, 28, 2011 | Mar, 28, 2011 |

Sang-Hee, Won

(Sign)

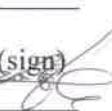
Mar. 25. 2011

Sang-Hee, Won / Quality Assurance

Date

Study Personnel and Participants

The test participants recognized the study plan, manual, procedure, guide of Marine Bio-industry Development Center in performing the test.

| | Name | Date |
|---------------------------------|---|---------------|
| Study Personnel | Jae-Young, Baek (sign)  | Mar. 25, 2011 |
| | Jae-Woo, Lee (sign)  | Mar. 25, 2011 |
| | Ka-na, Song (sign)  | Mar. 25, 2011 |
| | Eun-Jung, Jung (sign)  | Mar. 25, 2011 |
| QA/QC | Yeon Su, Park (sign)  | Mar. 25, 2011 |
| Efficacy Testing Manager | Byeong-Jin Lee (sign)  | Mar. 25, 2011 |

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1. Results

1.1 Loading record

| | |
|-------------|---------------|
| Vessel name | HANJIN DURBAN |
| IMO number | 9375513 |

| | Water ballast tanks | | Sample WGT(Mt) | Loading (Date/Location) | Discharge (Date/Location) |
|---------|---------------------|---|----------------|-------------------------|-----------------------------------|
| Control | No.4 D/B W.B.T | P | 320 | Dec, 26 Pusan | Jan, 01. 2011 47° -59N,178-19E |
| | No.5 D/B W.B.T | P | 544 | Dec, 26 Pusan | Jan, 01. 2011 47° -59N,178-19E |
| Treated | No.4 D/B W.B.T | P | 320 | Dec, 26 Pusan | Jan, 01. 2011 47° -59N,177-39E |
| | No.5 D/B W.B.T | S | 544 | Dec, 26 Pusan | Jan, 01. 2011 47° -59N,177-39E |

1.2 Water parameters

Table 1. Water parameters at field

| Date | Sample ID | pH | Temp (°C) | Salinity (%) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------|------------|--------------|-----------|-----------------|--------------|
| Day 0 | OwS | 7.40±0.00 | 13.79±0.00 | 35.14±0.00 | 6.03±0.01 | 7.8±0.0 | 0.624±0.000 |
| | OwM | 7.73±0.00 | 10.58±0.01 | 34.96±0.00 | 6.02±0.00 | 4.2±0.1 | 0.631±0.000 |
| | OwE | 7.65±0.00 | 10.12±0.00 | 35.12±0.01 | 5.98±0.01 | 4.1±0.0 | 0.626±0.000 |
| | T0S | 8.14±0.00 | 10.63±0.00 | 35.19±0.01 | 6.06±0.00 | 3.7±0.0 | 0.000±0.000 |
| | T0M | 7.45±0.01 | 11.99±0.00 | 21.72±0.01 | 7.56±0.01 | 1.0±0.1 | 0.000±0.000 |
| | T0E | 7.51±0.00 | 11.96±0.00 | 22.13±0.00 | 7.35±0.01 | 1.3±0.1 | 0.000±0.000 |
| Day 6 | C6S | 7.67±0.01 | 7.75±0.01 | 35.08±0.00 | 6.01±0.00 | 18.4±0.2 | 0.104±0.000 |
| | C6M | 7.85±0.00 | 7.84±0.00 | 35.08±0.00 | 6.11±0.01 | 17.8±0.0 | 0.109±0.000 |
| | C6E | 7.62±0.01 | 7.59±0.00 | 35.10±0.00 | 6.06±0.01 | 18.0±0.0 | 0.108±0.000 |
| | T6/S1 | 8.64±0.01 | 9.15±0.01 | 34.90±0.00 | 5.80±0.01 | 173±0.2 | 0.000±0.000 |
| | T6/S2 | 8.49±0.00 | 8.69±0.00 | 34.82±0.01 | 5.72±0.00 | 172±0.1 | 0.000±0.000 |
| | T6/S3 | 8.51±0.00 | 9.16±0.02 | 34.84±0.03 | 5.73±0.00 | 14.9±0.1 | 0.000±0.000 |
| | T6/M1 | 8.69±0.00 | 9.14±0.00 | 34.91±0.01 | 5.79±0.01 | 15.5±0.2 | 0.000±0.000 |
| | T6/M2 | 8.34±0.00 | 8.67±0.00 | 34.87±0.01 | 5.84±0.00 | 18.5±0.1 | 0.000±0.000 |
| | T6/M3 | 8.30±0.00 | 9.11±0.00 | 34.89±0.01 | 5.72±0.01 | 25.4±0.2 | 0.000±0.000 |
| | T6/E1 | 8.43±0.00 | 9.13±0.01 | 34.94±0.02 | 5.86±0.01 | 19.5±0.2 | 0.000±0.000 |
| | T6/E2 | 8.22±0.01 | 9.00±0.00 | 34.93±0.01 | 5.89±0.02 | 20.5±0.2 | 0.000±0.000 |
| | T6/E3 | 8.07±0.01 | 9.20±0.01 | 35.01±0.01 | 5.82±0.01 | 20.6±0.2 | 0.000±0.000 |

Data were presented as mean±S.D of three repeated measurement.

Table 2. Results of DOC, POC, TSS

| Date | Sample ID | TOC ^{a)} (mg/L) | DOC ^{b)} (mg/L) | POC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | 3.04±0.04 | 1.87±0.04 | 1.17 | 9.3±0.6 |
| | OwM | 3.07±0.01 | 1.86±0.02 | 1.21 | 8.7±0.3 |
| | OwE | 3.06±0.02 | 1.89±0.02 | 1.17 | 9.1±0.3 |

Data were presented as mean±S.D of three repeated measurement.

a) TOC: Total Organic Carbon

b) DOC: Dissolved Organic Carbon

c) POC: Particulate Organic Carbon (POC=TOC-DOC)

d) TSS: Total Suspended Solids

Table 3. TRC decay

| Date | Measurement time | Control | Treated |
|--------------|------------------|----------------|----------------|
| | | TRC con. (ppm) | TRC con. (ppm) |
| Day 0 | 18:00 | 0.02±0.01 | 2.89±0.03 |
| Day 1 | 14:00 | 0.07±0.03 | 1.78±0.04 |
| Day 2 | 14:00 | 0.03±0.01 | 0.76±0.07 |
| Day 3 | 14:00 | 0.04±0.01 | 0.33±0.05 |
| Day 4 | 14:00 | 0.05±0.01 | 0.24±0.02 |
| Day 5 | 14:00 | 0.04±0.00 | 0.10±0.02 |
| Day 6 | 16:00 | 0.03±0.01 | 0.04±0.01 |

Data were presented as mean±S.D of three repeated measurement.

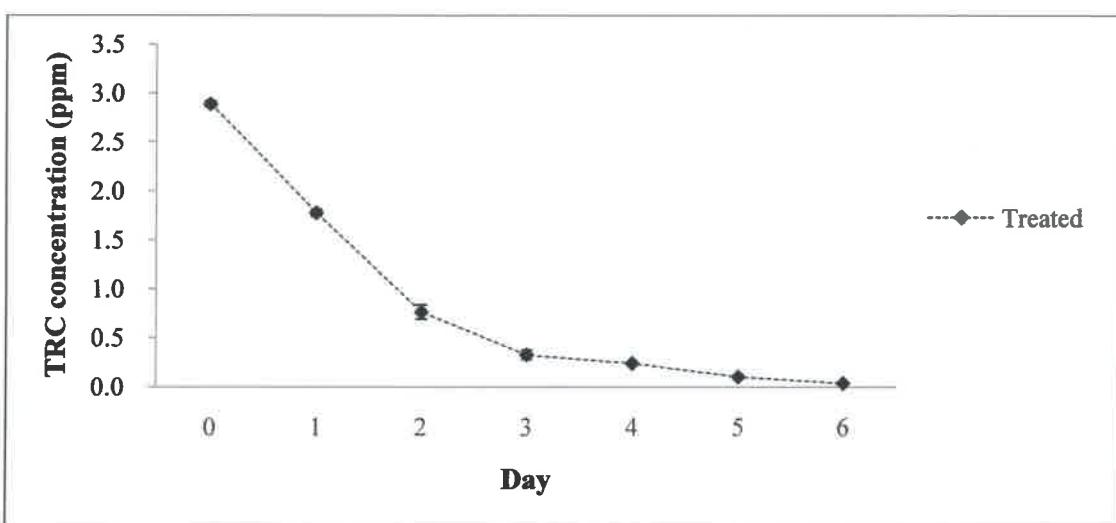


Figure 1. Change of TRC concentration of treated ballast seawater for 7 days

1.3 Influent water : Original water

1) $\geq 50 \mu\text{m}$ organism

Table 4. Survival number of the organism larger than $50 \mu\text{m}$

| Sampling | Phyla/Divisions | Species | Survival number/ m^3 | Minimum Dimension (μm) |
|---------------|-----------------------------|--------------------------|---|-------------------------------------|
| Start | Arthropoda | <i>Oithona</i> sp. | 2160 ± 1080 | 120 ~ 220 |
| | | <i>Metridia</i> sp. | 630 ± 156 | 190 ~ 220 |
| | Podonidae | <i>Podon</i> sp. | 360 ± 312 | 55 ~ 80 |
| | Chordata | <i>Oikopleura</i> sp. | 180 ± 156 | 160 ~ 250 |
| | Heterokontophyta | <i>Coscinodiscus</i> sp. | 12960 ± 1686 | 60 ~ 120 |
| Middle | Arthropoda | <i>Oithona</i> sp. | 2320 ± 870 | 120 ~ 220 |
| | | <i>Metridia</i> sp. | 387 ± 443 | 190 ~ 220 |
| | Podonidae | <i>Podon</i> sp. | 193 ± 167 | 55 ~ 80 |
| | Heterokontophyta | <i>Coscinodiscus</i> sp. | 20203 ± 932 | 60 ~ 120 |
| End | Arthropoda | <i>Oithona</i> sp. | 2610 ± 312 | 120 ~ 220 |
| | | <i>Metridia</i> sp. | 450 ± 412 | 190 ~ 220 |
| | Chordata | <i>Oikopleura</i> sp. | 90 ± 156 | 160 ~ 250 |
| | Heterokontophyta | <i>Coscinodiscus</i> sp. | 13410 ± 3725 | 60 ~ 120 |
| Total | 5 species 4 phyla/divisions | | 18651 ± 3858 | - |
| Acceptability | | | Influent condition: Acceptable ($\geq 10^2$ ind./ m^3) | - |

Data were presented as mean \pm S.D of three repeated measurement.
Some species were rare, the standard deviation was high.

2) 10 µm - 50 µm organism

Table 5. Survival number of the organism between 10 to 50 µm

| Sampling | Phyla/Divisions | Species | Survival number/mL (Mean) | Minimum Dimension (µm) |
|---------------|-----------------------------|----------------------------|---|------------------------|
| Start | Heterokontophyta | <i>Thalassiosira</i> sp. | 424 ± 52 | 15 ~ 25 |
| | | <i>Skeletonema</i> sp. | 29 ± 3 | 11 ~ 15 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 290 ± 63 | 12 ~ 13 |
| | Biddulphiaceae | <i>Eucampia</i> sp. | 21 ± 5 | 20 ~ 40 |
| Middle | Heterokontophyta | <i>Thalassiosira</i> sp. | 417 ± 17 | 15 ~ 25 |
| | | <i>Melosira</i> sp. | 13 ± 2 | 20 ~ 30 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 256 ± 36 | 12 ~ 13 |
| | Dinophyta | <i>Protoperidinium</i> sp. | 39 ± 3 | 15 ~ 20 |
| End | Heterokontophyta | <i>Thalassiosira</i> sp. | 327 ± 31 | 15 ~ 25 |
| | | <i>Skeletonema</i> sp. | 30 ± 3 | 11 ~ 15 |
| | Chlorodendraceae | <i>Tetraselmis</i> sp. | 285 ± 11 | 12 ~ 13 |
| | Dinophyta | <i>Ceratium</i> sp. | 14 ± 3 | 35 ~ 45 |
| Total | 7 species 3 phyla/divisions | | 715 ± 71 | - |
| Acceptability | | | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |

Data were presented as mean±S.D of three repeated measurement.
Some species were rare, the standard deviation was high..

1.4 Elimination efficacy

1) $\geq 50 \mu\text{m}$ organism

Table 6. Survival number of the organism larger than $50 \mu\text{m}$

| Test substance | Sampling | Survival number/ m^3 | Acceptability | Elimination efficacy (%) |
|---|---------------|---|---|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 16290 \pm 2887 | Influent condition: Acceptable ($\geq 10^2 \text{ ind./m}^3$) | - |
| | | M ^{b)} 23103 \pm 1098 | | |
| | | E ^{c)} 16560 \pm 3299 | | |
| Control (untreated) seawater | Day 6 (C6) | S 8010 \pm 1627 | Effluent condition: Acceptable ($\geq 10 \text{ ind./m}^3$) | - |
| | | S 4237 \pm 645 | | |
| | | E 4857 \pm 2110 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 \pm 0 | - | 100.0 ^{d)} |
| | | M 0 \pm 0 | | |
| | | E 0 \pm 0 | | |
| | Day 6 (T6) | S1 0 \pm 0 | Effluent condition: Acceptable (<10 ind./ m^3) | 100.0 ^{e)} |
| | | S2 0 \pm 0 | | |
| | | S3 0 \pm 0 | | |
| | | M1 0 \pm 0 | | |
| | | M2 0 \pm 0 | | |
| | | M3 0 \pm 0 | | |
| | | E1 0 \pm 0 | | |
| | | E2 0 \pm 0 | | |
| | | E3 0 \pm 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean \pm S.D of three repeated measurement.

2) 10 µm - 50 µm organism

Table 7. Survival number of the organism between 10 and 50 µm

| Test substance | Sampling | Survival number/mL | | Acceptability | Elimination efficacy (%) |
|---|---------------|--------------------|----------|---|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 764 ± 98 | Influent condition: Acceptable ($\geq 10^2$ ind./mL) | - |
| | | M ^{b)} | 726 ± 21 | | |
| | | E ^{c)} | 656 ± 37 | | |
| Control (untreated) seawater | Day 6 (C6) | S | 109 ± 6 | Effluent condition: Acceptable (≥ 10 ind./mL) | - |
| | | M | 141 ± 2 | | |
| | | E | 105 ± 3 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 6 (T6) | S1 | 0 ± 0 | Effluent condition: Acceptable (<10 ind./mL) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

3) Heterotrophic bacteria

Table 8. Survival number of heterotrophic bacteria

| Test substance | Sampling | CFU / mL | Acceptability | Elimination efficacy (%) |
|------------------------------------|---------------|-----------------------------|---------------|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 100 ± 10 | - | - |
| | | M ^{b)} 140 ± 36 | | |
| | | E ^{c)} 200 ± 10 | | |
| Control (untreated) seawater | Day 6 (C6) | S 2487 ± 93 | - | - |
| | | M 3093 ± 315 | | |
| | | E 3203 ± 215 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 ± 0 | - | 100.0 ^{d)} |
| | | M 0 ± 0 | | |
| | | E 0 ± 0 | | |
| | Day 6 (T6) | S1 0 ± 0 | - | 100.0 ^{e)} |
| | | S2 0 ± 0 | | |
| | | S3 0 ± 0 | | |
| | | M1 0 ± 0 | | |
| | | M2 0 ± 0 | | |
| | | M3 0 ± 0 | | |
| | | E1 0 ± 0 | | |
| | | E2 0 ± 0 | | |
| | | E3 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

4) *Escherichia coli***Table 9. Survival number of *Escherichia coli***

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|------------------------------|------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 3 ± 1 | - | - |
| | | M ^{b)} | 4 ± 2 | | |
| | | E ^{c)} | 3 ± 1 | | |
| Control (untreated) seawater | Day 6 (C6) | S | 0 ± 0 | - | - |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 1 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | 100.0 ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 6 (T6) | S1 | 0 ± 0 | Effluent condition: Acceptable (<250 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

5) *Enterococcus faecalis***Table 10. Survival number of *Enterococcus faecalis***

| Test substance | Sampling | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|---|---------------|--------------------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} 2 ± 0 | - | - |
| | | M ^{b)} 0 ± 1 | | |
| | | E ^{c)} 0 ± 0 | | |
| Control (untreated) seawater | Day 6 (C6) | S 0 ± 0 | - | - |
| | | M 0 ± 0 | | |
| | | E 0 ± 1 | | |
| Treated ballast seawater | Day 0 (T0) | S 0 ± 0 | - | 100.0 ^{d)} |
| | | M 0 ± 0 | | |
| | | E 0 ± 0 | | |
| | Day 6 (T6) | S1 0 ± 0 | Effluent condition: Acceptable (<100 CFU/ 100ml) | 100.0 ^{e)} |
| | | S2 0 ± 0 | | |
| | | S3 0 ± 0 | | |
| | | M1 0 ± 0 | | |
| | | M2 0 ± 0 | | |
| | | M3 0 ± 0 | | |
| | | E1 0 ± 0 | | |
| | | E2 0 ± 0 | | |
| | | E3 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) Calculation = (Ow-T0)/Ow*100, e) Calculation = (Ow-T5)/Ow*100

Data were presented as mean±S.D of three repeated measurement.

6) *Vibrio cholera* O1, O139**Table 11. Survival number of *Vibrio cholera* O1, O139**

| Test substance | Sampling | | CFU / 100 mL | Acceptability | Elimination efficacy (%) |
|------------------------------------|---------------|-----------------|--------------|--|--------------------------|
| Original water | Day 0 (Ow) | S ^{a)} | 0 ± 0 | - | - |
| | | M ^{b)} | 0 ± 0 | | |
| | | E ^{c)} | 0 ± 0 | | |
| Control (untreated) seawater | Day 6 (C6) | S | 0 ± 0 | - | - |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| Treated ballast seawater | Day 0 (T0) | S | 0 ± 0 | - | N.D ^{d)} |
| | | M | 0 ± 0 | | |
| | | E | 0 ± 0 | | |
| | Day 6 (T6) | S1 | 0 ± 0 | Effluent condition: Acceptable (<1 CFU/ 100ml) | N.D |
| | | S2 | 0 ± 0 | | |
| | | S3 | 0 ± 0 | | |
| | | M1 | 0 ± 0 | | |
| | | M2 | 0 ± 0 | | |
| | | M3 | 0 ± 0 | | |
| | | E1 | 0 ± 0 | | |
| | | E2 | 0 ± 0 | | |
| | | E3 | 0 ± 0 | | |

a) S: Start, b) M: Middle c) E: End, d) N.D: Not detected

Data were presented as mean±S.D of three repeated measurement.

2. Validity

2.1 Water parameters measurement

- All measurements were tested more than three times.
- Water parameters were analyzed as soon as possible after sampling.

2.2 Biological efficacy test

- Influent condition in all tests was acceptable for IMO standards (MEPC58/2/4).
 - The organism larger than 50 µm: $\geq 10^2$ individuals/m³
 - The organism between 10 µm and 50 µm: $\geq 10^2$ individuals / mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| OwS | 16290 ± 2887 | 764 ± 98 |
| OwM | 23103 ± 1098 | 726 ± 21 |
| OwE | 16560 ± 3299 | 656 ± 37 |

- Discharge results from the control water were a concentration more than the values in regulation D2.1.
 - The organism larger than 50 µm: ≥ 10 individuals/m³
 - The organism between 10 µm and 50 µm: ≥ 10 individuals/mL

| Sample | $\geq 50 \mu\text{m}$ (individuals/m ³) | $10 \mu\text{m} - 50 \mu\text{m}$ (individuals/mL) |
|--------|--|---|
| C6S | 8010 ± 1124 | 109 ± 6 |
| C6M | 4237 ± 645 | 141 ± 2 |
| C6E | 4857 ± 2110 | 105 ± 3 |

- The samples should be analysed as soon as possible after sampling.

| Date | Sample | Sampling time | Test ending time |
|-------|-------------|---------------|------------------|
| Day 0 | OwS | 16:19 | 24:00 |
| | OwM | 16:30 | 24:00 |
| | OwE | 16:40 | 24:00 |
| | T0S | 16:19 | 24:00 |
| | T0M | 16:30 | 24:00 |
| | T0E | 16:40 | 24:00 |
| Day 6 | C6S | 15:07 | 20:30 |
| | C6M | 15:26 | 20:30 |
| | C6E | 15:46 | 20:30 |
| | T6/S1, 2, 3 | 13:50 | 20:30 |
| | T6/M1, 2, 3 | 14:05 | 20:30 |
| | T6/E1, 2, 3 | 14:30 | 20:30 |

3. Conclusion

3.1 Biological efficacy in treated ballast seawater by PurimarTM BWMS

- Treated ballast seawater by PurimarTM BWMS was capable of eliminating organism larger than 50 µm with an efficiency of 100%.
- Treated ballast seawater water by PurimarTM BWMS was capable of eliminating organism between 10 and 50 µm with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating heterotrophic bacteria with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating *Escherichia coli* with an efficiency of 100 %.
- Treated ballast seawater by PurimarTM BWMS was capable of eliminating *Enterococcus faecalis* with an efficiency of 100 %.
- *Vibrio cholera* O1, O139 were not detected.
- **Therefore, treated ballast seawater by PurimarTM BWMS was capable of removing zooplankton, phytoplankton, bacteria. And PurimarTM BWMS showed discharge of treated ballast water in compliance with regulation D-2.**

4. Appendix

Appendix 1. Water parameters at field

| Date | Sample ID | R ^{a)} | pH | Temp (°C) | Salinity (%) | DO (mg/L) | Turbidity (NTU) | Chlorophylla |
|-------|-----------|-----------------|------|-----------|--------------|-----------|-----------------|--------------|
| Day 0 | OwS | R1 | 7.40 | 13.79 | 35.14 | 6.04 | 7.8 | 0.624 |
| | | R2 | 7.40 | 13.79 | 35.14 | 6.02 | 7.8 | 0.624 |
| | | R3 | 7.40 | 13.79 | 35.14 | 6.02 | 7.8 | 0.624 |
| | OwM | R1 | 7.73 | 10.58 | 34.96 | 6.02 | 4.1 | 0.631 |
| | | R2 | 7.73 | 10.58 | 34.96 | 6.02 | 4.2 | 0.631 |
| | | R3 | 7.73 | 10.59 | 34.96 | 6.02 | 4.2 | 0.631 |
| | OwE | R1 | 7.65 | 10.12 | 35.12 | 5.99 | 4.1 | 0.626 |
| | | R2 | 7.65 | 10.12 | 35.12 | 5.98 | 4.1 | 0.626 |
| | | R3 | 7.65 | 10.12 | 35.13 | 5.98 | 4.1 | 0.626 |
| | T0S | R1 | 8.14 | 10.63 | 35.19 | 6.06 | 3.7 | 0.000 |
| | | R2 | 8.14 | 10.63 | 35.19 | 6.06 | 3.7 | 0.000 |
| | | R3 | 8.14 | 10.63 | 35.18 | 6.06 | 3.7 | 0.000 |
| | T0M | R1 | 8.20 | 10.31 | 35.12 | 6.08 | 8.9 | 0.000 |
| | | R2 | 8.21 | 10.31 | 35.13 | 6.07 | 8.9 | 0.000 |
| | | R3 | 8.20 | 10.31 | 35.12 | 6.07 | 9.0 | 0.000 |
| | T0E | R1 | 8.35 | 10.36 | 35.11 | 6.11 | 3.6 | 0.000 |
| | | R2 | 8.35 | 10.36 | 35.11 | 6.10 | 3.7 | 0.000 |
| | | R3 | 8.35 | 10.36 | 35.11 | 6.09 | 3.6 | 0.000 |

a) R: Replicate

| Date | Sample ID & lot No. | R ^{a)} | pH | Temp (°C) | Salinity (‰) | DO (mg/L) | Turbidity (NTU) | Chlorophyla |
|-------|---------------------|-----------------|------|-----------|--------------|-----------|-----------------|-------------|
| Day 6 | C6S | R1 | 7.67 | 7.74 | 35.08 | 6.01 | 18.5 | 0.104 |
| | | R2 | 7.67 | 7.75 | 35.08 | 6.01 | 18.5 | 0.104 |
| | | R3 | 7.68 | 7.75 | 35.08 | 6.01 | 18.1 | 0.104 |
| | C6M | R1 | 7.85 | 7.84 | 35.08 | 6.12 | 17.8 | 0.109 |
| | | R2 | 7.85 | 7.84 | 35.08 | 6.12 | 17.8 | 0.109 |
| | | R3 | 7.85 | 7.84 | 35.08 | 6.10 | 17.8 | 0.109 |
| | C6E | R1 | 7.62 | 7.59 | 35.10 | 6.07 | 18.0 | 0.108 |
| | | R2 | 7.62 | 7.59 | 35.10 | 6.05 | 18.0 | 0.108 |
| | | R3 | 7.63 | 7.59 | 35.10 | 6.05 | 18.0 | 0.108 |
| | T6/S1 | R1 | 8.65 | 9.15 | 34.90 | 5.81 | 17.5 | 0.000 |
| | | R2 | 8.64 | 9.15 | 34.90 | 5.80 | 17.2 | 0.000 |
| | | R3 | 8.64 | 9.16 | 34.90 | 5.80 | 17.1 | 0.000 |
| | T6/S2 | R1 | 8.49 | 8.69 | 34.82 | 5.72 | 17.3 | 0.000 |
| | | R2 | 8.49 | 8.69 | 34.82 | 5.72 | 17.1 | 0.000 |
| | | R3 | 8.49 | 8.69 | 34.81 | 5.72 | 17.3 | 0.000 |
| | T6/S3 | R1 | 8.51 | 9.14 | 34.81 | 5.73 | 15.0 | 0.000 |
| | | R2 | 8.51 | 9.18 | 34.84 | 5.73 | 14.8 | 0.000 |
| | | R3 | 8.51 | 9.17 | 34.87 | 5.73 | 14.8 | 0.000 |
| | T6/M1 | R1 | 8.69 | 9.14 | 34.90 | 5.79 | 15.6 | 0.000 |
| | | R2 | 8.69 | 9.14 | 34.91 | 5.79 | 15.6 | 0.000 |
| | | R3 | 8.69 | 9.14 | 34.91 | 5.78 | 15.3 | 0.000 |
| | T6/M2 | R1 | 8.34 | 8.67 | 34.87 | 5.84 | 18.5 | 0.000 |
| | | R2 | 8.34 | 8.67 | 34.87 | 5.84 | 18.5 | 0.000 |
| | | R3 | 8.34 | 8.67 | 34.86 | 5.84 | 18.6 | 0.000 |
| | T6/M3 | R1 | 8.30 | 9.11 | 34.88 | 5.73 | 25.2 | 0.000 |
| | | R2 | 8.30 | 9.11 | 34.89 | 5.71 | 25.4 | 0.000 |
| | | R3 | 8.30 | 9.11 | 34.89 | 5.71 | 25.6 | 0.000 |
| | T6/E1 | R1 | 8.43 | 9.12 | 34.96 | 5.87 | 19.3 | 0.000 |
| | | R2 | 8.43 | 9.13 | 34.93 | 5.86 | 19.6 | 0.000 |
| | | R3 | 8.43 | 9.13 | 34.93 | 5.85 | 19.7 | 0.000 |
| | T6/E2 | R1 | 8.21 | 9.00 | 34.93 | 5.91 | 20.7 | 0.000 |
| | | R2 | 8.22 | 9.00 | 34.93 | 5.88 | 20.3 | 0.000 |
| | | R3 | 8.22 | 9.00 | 34.92 | 5.87 | 20.5 | 0.000 |
| | T6/E3 | R1 | 8.06 | 9.20 | 35.01 | 5.83 | 20.5 | 0.000 |
| | | R2 | 8.07 | 9.20 | 35.02 | 5.82 | 20.5 | 0.000 |
| | | R3 | 8.07 | 9.19 | 35.01 | 5.82 | 20.8 | 0.000 |

Appendix 2. Water parameters (TOC, DOC, TSS) of original water (Ow)

| Date | Sample ID | R ^{a)} | TOC ^{b)} (mg/L) | DOC ^{c)} (mg/L) | TSS ^{d)} (mg/L) |
|-------|-----------|-----------------|-----------------------------|-----------------------------|-----------------------------|
| Day 0 | OwS | R1 | 3.07 | 1.91 | 8.6 |
| | | R2 | 3.05 | 1.87 | 9.8 |
| | | R3 | 3.00 | 1.83 | 9.6 |
| | OwM | R1 | 3.08 | 1.88 | 8.4 |
| | | R2 | 3.08 | 1.85 | 8.8 |
| | | R3 | 3.06 | 1.86 | 9.0 |
| | OwE | R1 | 3.08 | 1.89 | 9.0 |
| | | R2 | 3.06 | 1.87 | 8.8 |
| | | R3 | 3.04 | 1.91 | 9.4 |

a) R: Replicate

b) TOC: Total Organic Carbon (TOC=DOC+POC)

c) DOC: Dissolved Organic Carbon

d) TSS: Total Suspended Solids

Appendix 3. TRC data

| Date | Group | TRC concentration (ppm) | | | Mean | S.D. ^a |
|--------------|---------|-------------------------|------|------|------|-------------------|
| Day 0 | Control | 0.02 | 0.02 | 0.03 | 0.02 | 0.01 |
| | Treated | 2.92 | 2.89 | 2.86 | 2.89 | 0.03 |
| Day 1 | Control | 0.04 | 0.09 | 0.08 | 0.07 | 0.03 |
| | Treated | 1.75 | 1.82 | 1.76 | 1.78 | 0.04 |
| Day 2 | Control | 0.03 | 0.02 | 0.04 | 0.03 | 0.01 |
| | Treated | 0.82 | 0.79 | 0.68 | 0.76 | 0.07 |
| Day 3 | Control | 0.05 | 0.04 | 0.03 | 0.04 | 0.01 |
| | Treated | 0.31 | 0.29 | 0.38 | 0.33 | 0.05 |
| Day 4 | Control | 0.06 | 0.06 | 0.04 | 0.05 | 0.01 |
| | Treated | 0.25 | 0.22 | 0.25 | 0.24 | 0.02 |
| Day 5 | Control | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 |
| | Treated | 0.12 | 0.10 | 0.09 | 0.10 | 0.02 |
| Day 6 | Control | 0.03 | 0.04 | 0.02 | 0.03 | 0.01 |
| | Treated | 0.05 | 0.03 | 0.03 | 0.04 | 0.01 |

^a S.D: standard deviation

* N.D: Not detected

Appendix 4. Survival number of $\geq 50\mu\text{m}$ organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|--------------------------|-------------------------------|-------|-------|-------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Oithona</i> sp. | 2160 | 3240 | 1080 | 2160 | 1080 |
| | | <i>Metridia</i> sp. | 810 | 540 | 540 | 630 | 156 |
| | | <i>Podon</i> sp. | 540 | 540 | 0 | 360 | 312 |
| | | <i>Oikopleura</i> sp. | 270 | 0 | 270 | 180 | 156 |
| | OwM | <i>Coscinodiscus</i> sp. | 14310 | 13500 | 11070 | 12960 | 1686 |
| | | <i>Oithona</i> sp. | 2320 | 1450 | 3190 | 2320 | 870 |
| | | <i>Metridia</i> sp. | 870 | 0 | 290 | 387 | 443 |
| | | <i>Podon</i> sp. | 290 | 290 | 0 | 193 | 167 |
| | OwE | <i>Coscinodiscus</i> sp. | 19140 | 20590 | 20880 | 20203 | 932 |
| | | <i>Oithona</i> sp. | 2430 | 2970 | 2430 | 2610 | 312 |
| | | <i>Metridia</i> sp. | 540 | 810 | 0 | 450 | 412 |
| | | <i>Oikopleura</i> sp. | 270 | 0 | 0 | 90 | 156 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 5. Survival number of $\geq 50\mu\text{m}$ organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|--------------------------|-------------------------------|------|------|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 6 | C6S | <i>Oithona</i> sp. | 2160 | 1350 | 2700 | 2070 | 679 |
| | | <i>Metridia</i> sp. | 0 | 270 | 810 | 360 | 412 |
| | | <i>Podon</i> sp. | 270 | 270 | 540 | 360 | 156 |
| | | <i>Coscinodiscus</i> sp. | 4050 | 5940 | 5670 | 5220 | 1022 |
| | C6M | <i>Oithona</i> sp. | 930 | 1240 | 1240 | 1137 | 179 |
| | | <i>Metridia</i> sp. | 620 | 0 | 0 | 207 | 358 |
| | | <i>Podon</i> sp. | 0 | 310 | 0 | 103 | 179 |
| | | <i>Coscinodiscus</i> sp. | 3410 | 2170 | 2790 | 2790 | 620 |
| | C6E | <i>Oithona</i> sp. | 930 | 1240 | 620 | 930 | 310 |
| | | <i>Metridia</i> sp. | 620 | 0 | 0 | 207 | 358 |
| | | <i>Coscinodiscus</i> sp. | 4030 | 5270 | 1860 | 3720 | 1726 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 6. Survival number of $\geq 50\mu\text{m}$ organism in treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | Classification | Survival number/ m^3 | | | Mean | SD ^{b)} |
|-------|-----------|----------------|-------------------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | TOS | - | 0 | 0 | 0 | 0 | 0 |
| | TOM | - | 0 | 0 | 0 | 0 | 0 |
| | TOE | - | 0 | 0 | 0 | 0 | 0 |
| Day 6 | T6/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 7. Survival number of 10 μm - 50 μm organism in original water(Influent water)

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|-------|-----------|----------------------------|----------------------|-----|-----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | OwS | <i>Thalassiosira</i> sp. | 410 | 482 | 380 | 424 | 52 |
| | | <i>Skeletonema</i> sp. | 32 | 29 | 26 | 29 | 3 |
| | | <i>Tetraselmis</i> sp. | 220 | 342 | 307 | 290 | 63 |
| | | <i>Eucampia</i> sp. | 26 | 22 | 16 | 21 | 5 |
| | OwM | <i>Thalassiosira</i> sp. | 432 | 398 | 422 | 417 | 17 |
| | | <i>Melosira</i> sp. | 12 | 11 | 15 | 13 | 2 |
| | | <i>Tetraselmis</i> sp. | 216 | 287 | 266 | 256 | 36 |
| | | <i>Protoperidinium</i> sp. | 42 | 37 | 39 | 39 | 3 |
| | OwE | <i>Thalassiosira</i> sp. | 322 | 298 | 360 | 327 | 31 |
| | | <i>Skeletonema</i> sp. | 28 | 29 | 34 | 30 | 3 |
| | | <i>Tetraselmis</i> sp. | 297 | 275 | 283 | 285 | 11 |
| | | <i>Ceratium</i> sp. | 11 | 17 | 15 | 14 | 3 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

Appendix 8. Survival number of 10µm - 50µm organism in control (untreated) seawater

| Date | Sample ID | Classification | Survival number / ml | | | Mean | SD ^{b)} |
|--------------|-----------|--------------------------|----------------------|----|----|------|------------------|
| | | | R1 ^{a)} | R2 | R3 | | |
| Day 6 | C6S | <i>Thalassiosira</i> sp. | 51 | 54 | 60 | 55 | 5 |
| | | <i>Tetraselmis</i> sp. | 49 | 42 | 51 | 47 | 5 |
| | | <i>Eucampia</i> sp. | 7 | 9 | 5 | 7 | 2 |
| | C6M | <i>Thalassiosira</i> sp. | 75 | 79 | 71 | 75 | 4 |
| | | <i>Melosira</i> sp. | 8 | 8 | 11 | 9 | 2 |
| | | <i>Tetraselmis</i> sp. | 56 | 55 | 61 | 57 | 3 |
| | C6E | <i>Thalassiosira</i> sp. | 49 | 53 | 52 | 51 | 2 |
| | | <i>Tetraselmis</i> sp. | 46 | 49 | 47 | 47 | 2 |
| | | <i>Ceratium</i> sp. | 8 | 7 | 4 | 6 | 2 |

a) R: Replicate

b) S.D: Standard deviation

Some species were rare, the standard deviation was high.

**Appendix 9. Survival number of 10 μm - 50 μm organism in treated ballast seawater
by treatment of Purimar™ BWMS**

| Date | Sample ID | Classification | Survival number /mL | | | Mean | SD^{b)} |
|--------------|------------------|-----------------------|----------------------------|-----------|-----------|-------------|------------------------|
| | | | R1^{a)} | R2 | R3 | | |
| Day 0 | T0S | - | 0 | 0 | 0 | 0 | 0 |
| | T0M | - | 0 | 0 | 0 | 0 | 0 |
| | T0E | - | 0 | 0 | 0 | 0 | 0 |
| Day 6 | T6/S1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/S2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/S3 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/M3 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E1 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E2 | - | 0 | 0 | 0 | 0 | 0 |
| | T6/E3 | - | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 10. Number of heterotrophic bacteria in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/mL | | | Mean | SD ^{b)} | |
|-------|-----------|------------------|-------|-------|-------|------------------|-----|
| | | R1 ^{a)} | R2 | R3 | | | |
| Day 0 | Ow | Start | 90 | 100 | 110 | 100 | 10 |
| | | Middle | 100 | 150 | 170 | 140 | 36 |
| | | End | 190 | 200 | 210 | 200 | 10 |
| | T0 | Start | 0 | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 | 0 |
| Day 6 | C6 | S | 2,380 | 2,530 | 2,550 | 2,487 | 93 |
| | | M | 2,750 | 3,160 | 3,370 | 3,093 | 315 |
| | | E | 2,980 | 3,220 | 3,410 | 3,203 | 215 |
| | T6 | S1 | 0 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 11. Number of *Escherichia coli* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 2 | 3 | 4 | 3 |
| | | Middle | 2 | 4 | 6 | 4 |
| | | End | 3 | 3 | 4 | 3 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| | C6 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 0 | 0 |
| | | E | 0 | 0 | 1 | 0 |
| Day 6 | T6 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 12. Number of *Enterococcus faecalis* in original water, control (untreated) seawater and treated ballast seawater by treatment of Purimar™ BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|-------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 2 | 2 | 2 | 2 |
| | | Middle | 0 | 0 | 1 | 1 |
| | | End | 0 | 0 | 0 | 0 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day6 | C6 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 0 | 0 |
| | | E | 0 | 0 | 1 | 1 |
| | T6 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

a) R: Replicate

b) S.D: Standard deviation

Appendix 13. Number of *Vibrio cholera* O1, O139 in original water, control (untreated) seawater and treated ballast seawater by treatment of PurimarTM BWMS

| Date | Sample ID | CFU/100 mL | | | Mean | SD ^{b)} |
|--------------|-----------|------------------|----|----|------|------------------|
| | | R1 ^{a)} | R2 | R3 | | |
| Day 0 | Ow | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| | T0 | Start | 0 | 0 | 0 | 0 |
| | | Middle | 0 | 0 | 0 | 0 |
| | | End | 0 | 0 | 0 | 0 |
| Day 6 | C6 | S | 0 | 0 | 0 | 0 |
| | | M | 0 | 0 | 0 | 0 |
| | | E | 0 | 0 | 0 | 0 |
| | T6 | S1 | 0 | 0 | 0 | 0 |
| | | S2 | 0 | 0 | 0 | 0 |
| | | S3 | 0 | 0 | 0 | 0 |
| | | M1 | 0 | 0 | 0 | 0 |
| | | M2 | 0 | 0 | 0 | 0 |
| | | M3 | 0 | 0 | 0 | 0 |
| | | E1 | 0 | 0 | 0 | 0 |
| | | E2 | 0 | 0 | 0 | 0 |
| | | E3 | 0 | 0 | 0 | 0 |

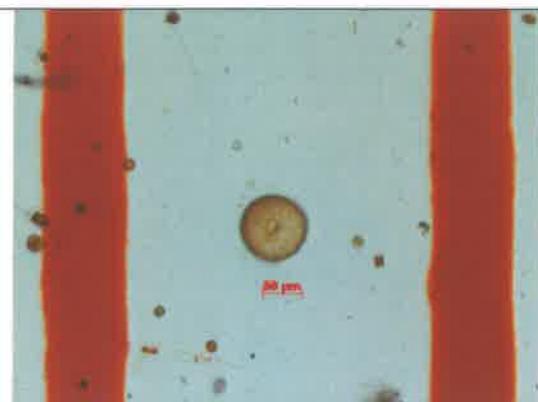
a) R: Replicate

b) S.D: Standard deviation

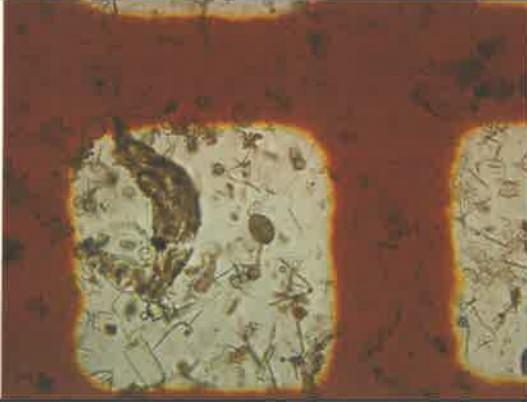
Appendix 14. The microscope image of $\geq 50\mu\text{m}$ in original water (Day0)

| Specific Name | Original water |
|-----------------------|--|
| <i>Oithona</i> sp. |  |
| <i>Metridia</i> sp. |  |
| <i>Podon</i> sp. |  |
| <i>Oikopleura</i> sp. |  |

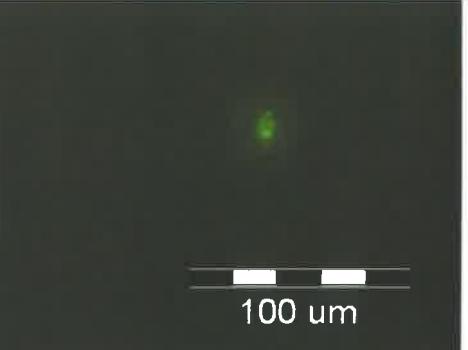
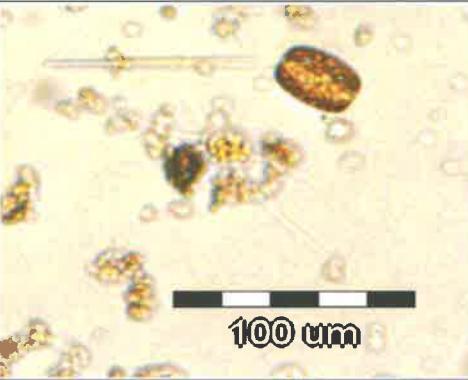
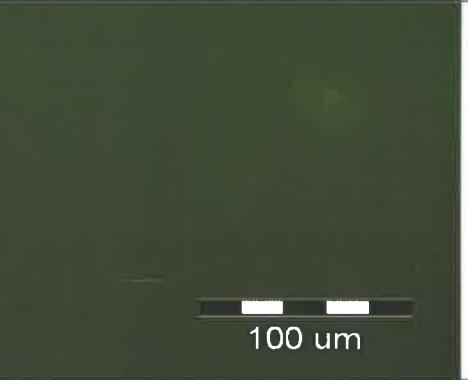
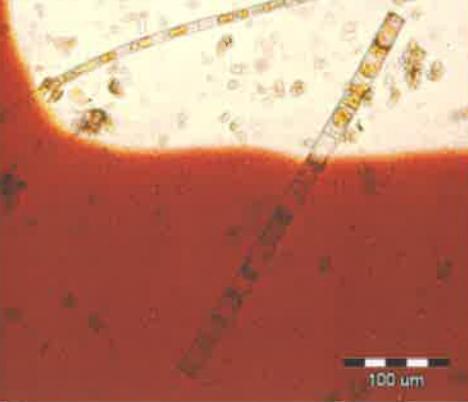
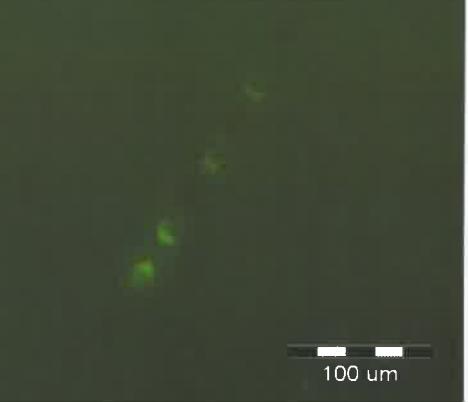
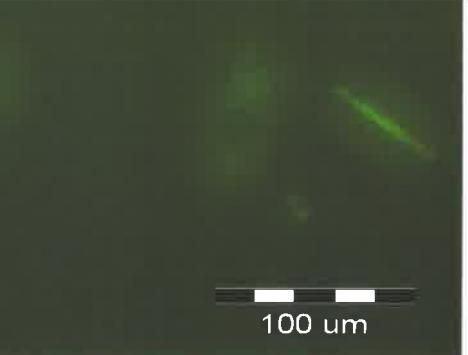
Coscinodiscus sp.

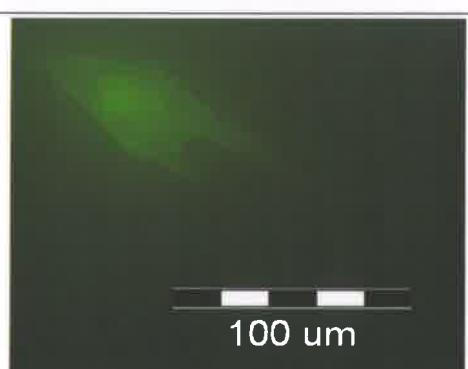
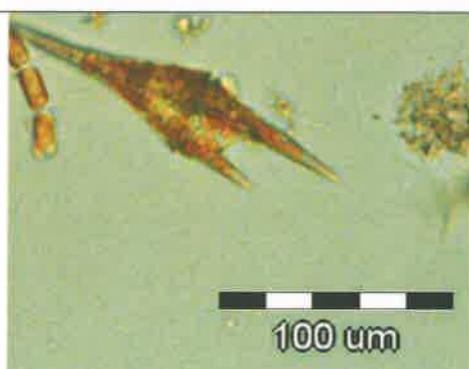
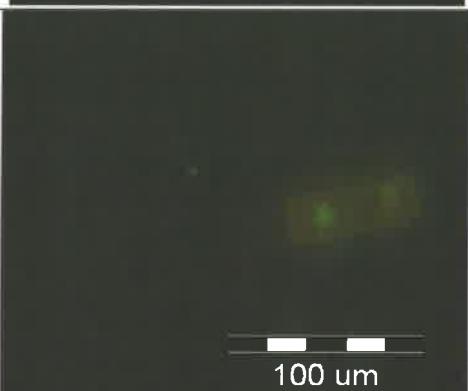
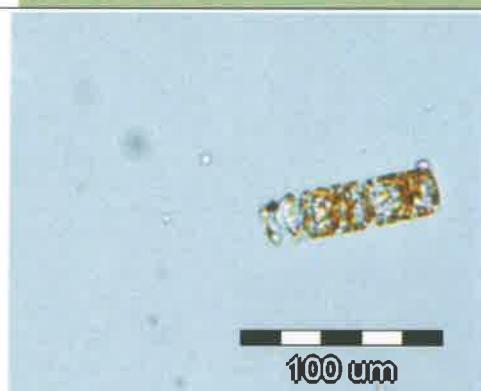
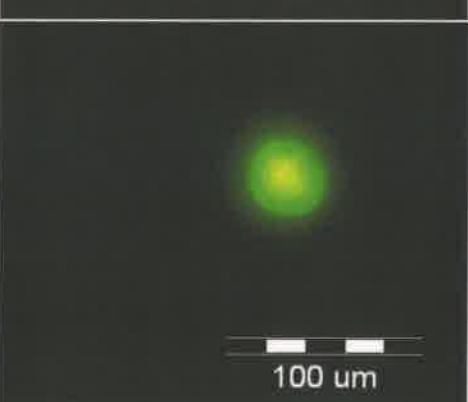
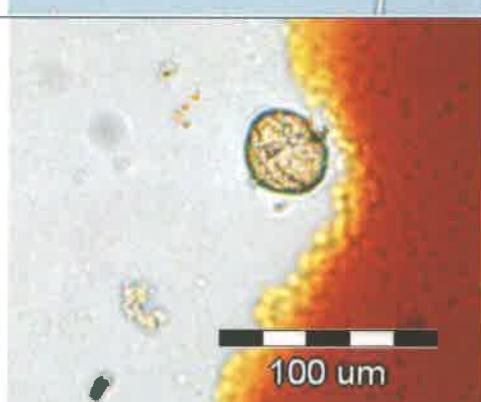


Appendix 15. The microscope image of $\geq 50\mu\text{m}$ in treated ballast seawater by treatment of PurimarTM BWMS (Day0)

| Specific Name | Control (untreated) seawater |
|----------------------|--|
| |  |

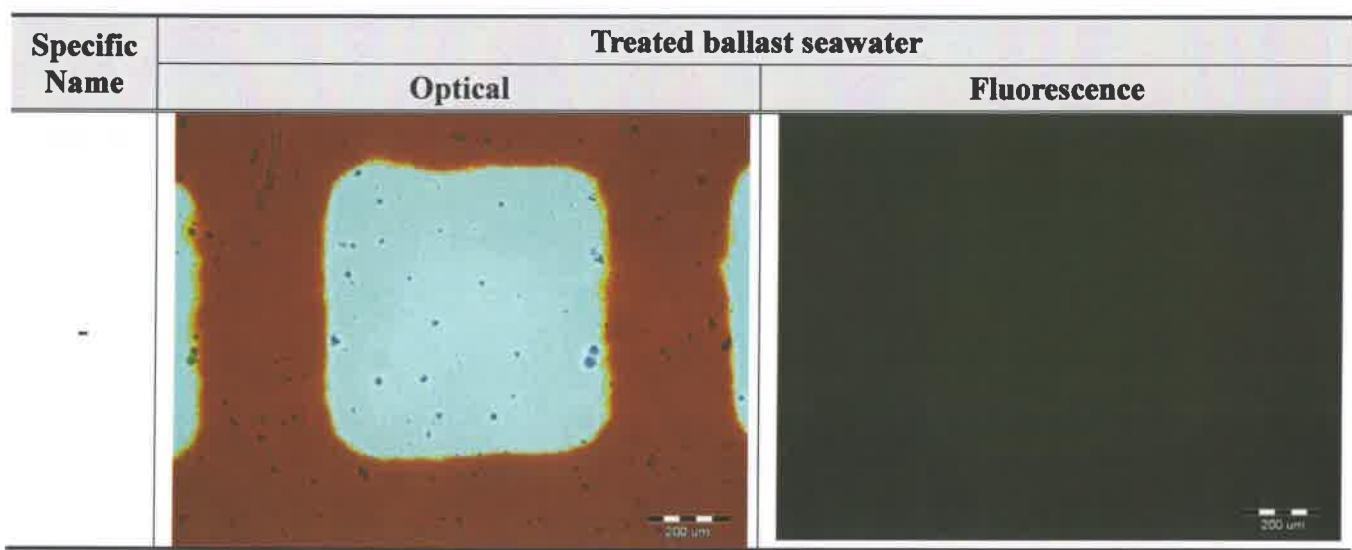
Appendix 16. The microscope image of $10\text{ }\mu\text{m}$ - $50\text{ }\mu\text{m}$ in original water

| Specific Name | Original water | |
|----------------------------|---|---|
| | Optical | Fluorescence |
| <i>Tetraselmis suecica</i> |  |  |
| <i>Thalassiosira sp.</i> |  |  |
| <i>Skeletonema sp.</i> |  |  |
| <i>Eucampia sp.</i> |  |  |

Ceratium sp.*Melosira sp.**Protoperidinium sp.*

Magnification : 200

Appendix 17. The microscope image of 10 μm –50 μm in treated ballast seawater by treatment of PurimarTM BWMS



Magnification : 200

Appendix 18. The image of heterotrophic bacteria: Day 0

Treated ballast seawater

Start



Middle



End



Original water

Start



Middle



End



Appendix 19. The image of heterotrophic bacteria: Day 6

Treated ballast seawater

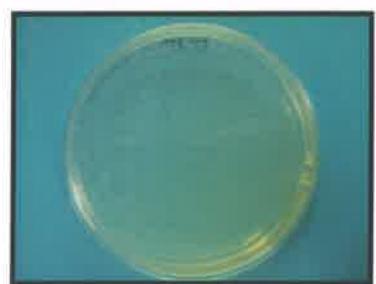
Strat(1)



Strat(2)



Strat(3)



Middle(1)



Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 20. The image of *Escherichia coli*: Day 0

Treated ballast seawater

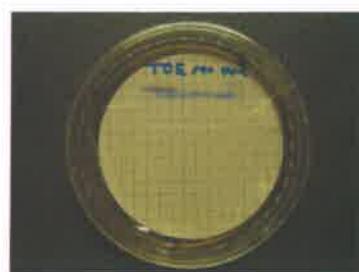
Start



Middle



End

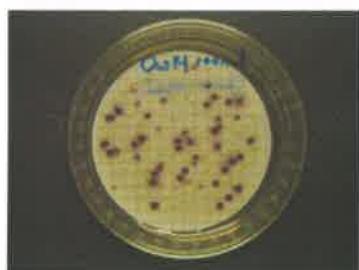


Original water

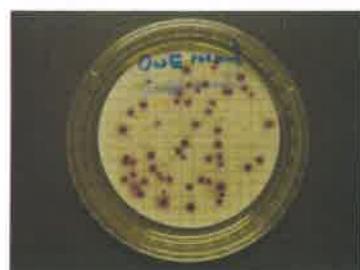
Start



Middle



End



Appendix 21. The image of *Escherichia coli*: Day 6

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 22. The image of *Enterococcus faecalis*: Day 0

Treated ballast seawater

Start



Middle



End



Original water

Start



Middle



End



Appendix 23. The image of *Enterococcus faecalis*: Day 6

Treated ballast seawater

Strat(1)



Strat(2)



Strat(3)



Middle(1)



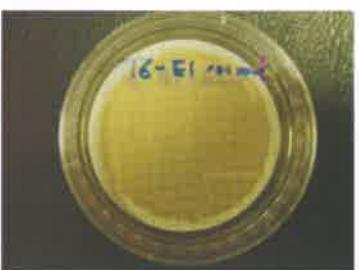
Middle(2)



Middle(3)



End(1)



End(2)



End(3)



Control (untreated) seawater

Start



Middle



End



Appendix 24. The image of *Vibrio cholera* O1, O139: Day 0

Original water

Start

Middle

End



Appendix 25. The image of *Vibrio cholera* O1, O139: Day 6

Control (untreated) seawater

Start

Middle

End



5. Attachment

- 5.1 Loading record
- 5.2 Sampling check & custody sheet
- 5.3 Freezing keeping sheet
- 5.4 Chain of custody record
- 5.5 Sample receipt form
- 5.6 Water parameter measurement sheet (I)
- 5.7 TOC measurement sheet
- 5.8 TSS(Total suspended solid) measurement sheet
- 5.9 TRC measurement sheet(For field)
- 5.10 Microbiology test sheet
- 5.11 Test results sheet with microbiology
- 5.12 Vibrio cholera O1, O139
- 5.13 Test results sheet – Counting
- 5.14 Test results sheet – Classification(II)